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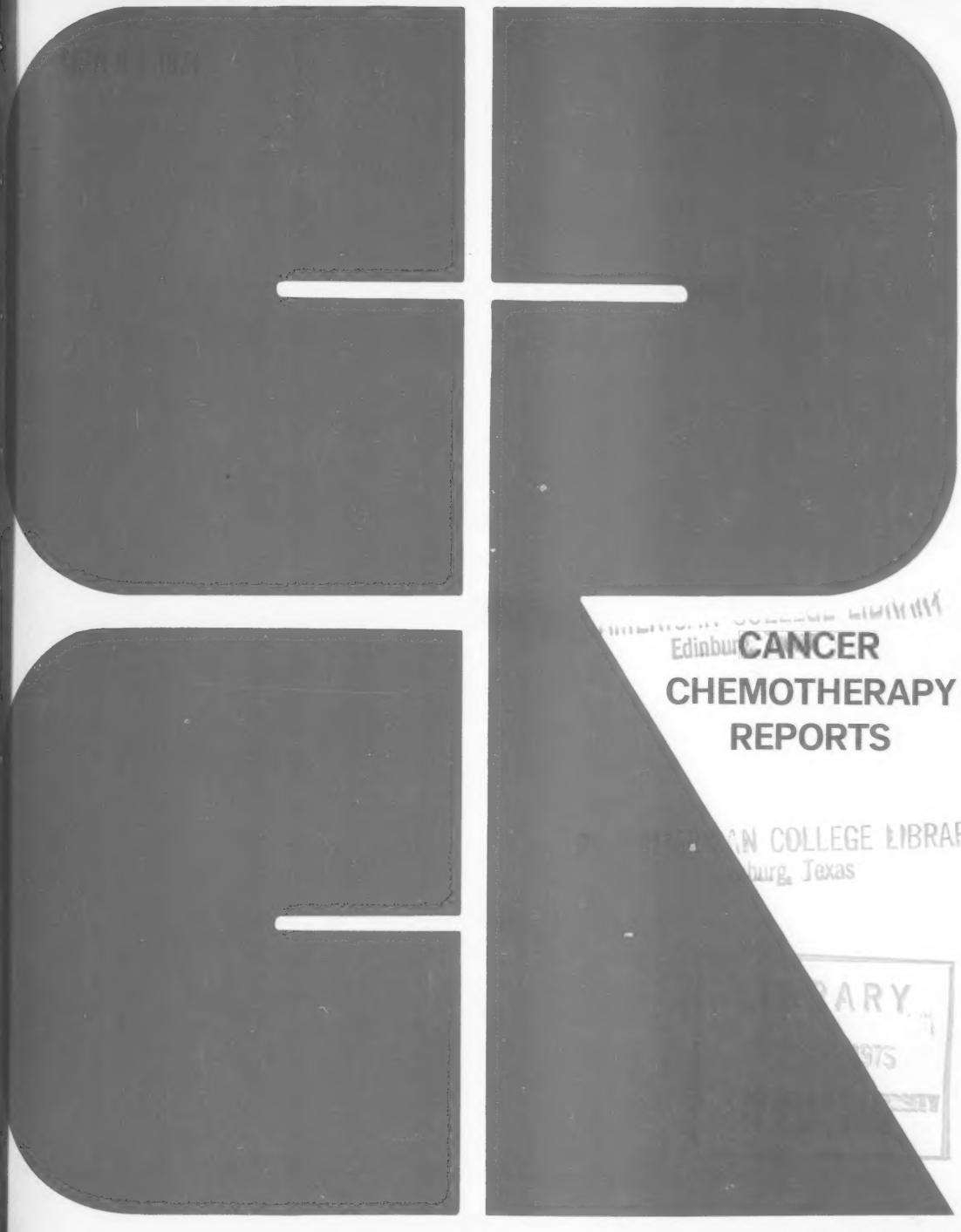

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The Secretary of Health, Education, and Welfare has determined that the publication of this periodical is necessary in the transaction of the public business required by law of this Department. Use of funds for printing this periodical has been approved by the Director of the Office of Management and Budget through November 30, 1973.

LETTER

An Evaluation of Procarbazine (NSC–77213) in the Treatment of Acute Leukemia [1]

In reference to your article by B.A. Chabner, R. Sponzo, S. Hubbard, et al, entitled "High-Dose Intermittent Intravenous Infusion of Procarbazine (NSC-77213)" (Cancer Chemother Rep 57:361–363, 1973), we would like to report our experiences with procarbazine in the treatment of acute lymphoblastic leukemia.

The Pediatric Division of the Southwest Oncology Group studied procarbazine in the treatment of children with acute lymphoblastic leukemia to determine if this drug had a therapeutic effectiveness of 25% or greater in producing a bone marrow remission. While procarbazine has been studied in a variety of cancers, reports of its therapeutic effectiveness in acute leukemia are limited (1,2). Eleven children with advanced leukemia that was resistant to standard therapy were given procarbazine at a dose level of 50–100 mg/m²/day orally. Some nausea and vomiting or decrease in appetite was reported in eight of the 11 children.

None of the 11 patients achieved a complete or partial remission. All patients had progression of their disease as reflected in an increase in percent of bone marrow blast cells, or an increase in the absolute number of circulating blast cells in the peripheral blood, or both. Two patients were considered unevaluable because of rapid progression of their disease which necessitated their removal from the study on the 7th day and 10th day, respectively. The remaining nine children had progression of their disease which necessitated removal from the study between the 15th and the 30th day. The probability of nine consecutive failures, if procarbazine is effective in 25% of the patients, is only 0.075.

These findings are similar to a previous report on eight patients of whom none achieved a remission and in which five cases were reported as evaluable (2).

[1] Procarbazine: CAS reg. No. 491-88-3; *p*-toluamide, *N*-isopropyl-α-(2-methylhydrazino)-, monohydrochloride.

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August 27, 1973

COMMENTARY

Adjuvant Temperature Effects in Cancer Therapy [1,2]

J. B. Block and C. G. Zubrod [3,4,5]

For almost 100 years there have been episodic observations that locally or systemically applied cold or heat to tumors will clinically influence tumor growth and produce regression. In general, these older observations appeared in the non-English literature and antedated the modern scientific observational approach. When heat or cold has been employed in the clinic, concomitant radiation and/or surgery was also often given in a generally uncontrolled manner. Thus, systemic heat therapy induced by bacterial vaccines, microwave diathermy, alternating currents, or hot baths for the treatment of cancer have come to be viewed as clinical experiences that are difficult to confirm, evaluate, or reproduce by peers. Yet, in this experience, which has largely been discarded from any modern critical evaluation, several authors have reported tumor regression in patients and animals with the use of applied heat alone.

Should this experience be assessed as at least indicating that profound temperature changes in or about a tumor may alter the growth characteristics of neoplasms in a manner that offers therapeutic potential? Induced temperature changes in normal tissue and in tumors may serve to modulate cell-cycle generation times and thus explain certain of these clinical observations. The purpose of this paper is to examine the evidence that applied heat or cold may influence the growth fraction of cancers in vivo. Many of the in vitro observations on the effects of heat on cancer will not be commented upon.

It is certainly a major conceptual advance in the field of cancer chemotherapy to appreciate that a tumor can be more responsive to an active drug's cytotoxic effects when the tumor is composed of a greater fraction of cells in a susceptible stage of the mitotic cycle (1–3). A natural outgrowth of this relationship between a drug's therapeutic potential and the cell-cycle stage of a tumor has been the development of modulators of cell-cycle activity. Several avenues of research are geared toward potentially modulating the cell cycle. These include the use of sex and other hormones and various vasoactive substances such as norepinephrine and the prostaglandins, the manipulation of cyclic adenosine monophosphate, and studies of a more fundamental nature. These concerns are directed to understanding processes basic to mechanisms of cellular growth and differentiation. However, such research does not preclude our having overlooked empirical or clinical observations such as temperature change which may have been relevant to cell-cycle modulation but were not understood or reproducible and were therefore discarded.

HEAT

There have been reports over the past several decades indicating that hyperthermia under proper conditions has deleterious growth effects on cancer (4–7). Recently, however, modern experimental models have been developed which unequivocally indicate that raising the thermal environment of a tumor upward relative to its surrounding normal tissues will cause selective toxic effects in vivo on neoplasms (8–11). The most divertingly in-

[1] Received June 26, 1973; accepted July 19, 1973.

[2] Chemical nomenclature, NSC numbers, and CAS registry numbers for all compounds mentioned in this paper are listed in the List of Compounds at the end of the text.

[3] Clinical Center, National Institutes of Health, Bethesda, Md.

[4] Division of Cancer Treatment, National Cancer Institute, Bethesda, Md.

[5] We would like to thank Dr. Theodore Kolobow for various discussions concerning this paper and Dr. Daniel Zaharko for providing the data for figures 1 and 2.

genious experiments have been those of Crile who injected Sarcoma 180 into the feet of Swiss mice and found that when the mice stood in hot water a striking regression of the established tumors was observed. More classic, but no less dramatic, has been the recent work by Popovic et al who transplanted the human Toolan No. IU tumor cell line into the hamster cheek pouch. While maintaining the hamster at systemic normothermic or hypothermic conditions, these authors showed that locally induced tumor hyperthermia, either absolute or relative to systemic temperature, caused tumor regression. Mouse foot tumors and hamster cheek pouches may not offer legitimate parallels to a human tumor, therefore, the results of Muckle et al may have more ready general appeal (12,13). They implanted the V × 2 carcinoma into the thigh of rabbits and demonstrated again that local (in vivo) hyperthermia of transplanted tumors produces impressive tumor resolution per se and prolongation of survival as compared to unheated controls.

Table 1 briefly lists the experimental tumor systems reported in the English literature in which an imposed elevated tumor temperature, relative to surrounding normal tissues, resulted in tumor regression. In addition, clinical studies where regression of tumors was caused by heat or where heat as an adjuvant to chemotherapy was claimed to produce an enhanced effect are also noted. The well-known lethal effects of heat on in vitro tumor systems are not included in this table, since temperatures greater than 41 C are usually required and, therefore, probably do not apply to potential clinical use. The data of these investigators indicate that one could experimentally evaluate the important features of potential interrelationships between elevated temperatures of normal and cancerous tissues and their response to agents producing tumor regression.

TABLE 1.—Tumors in which heat alone produced regression in vivo

Tumor system	Reference
S91 mouse melanoma	11, 14
Toolan human adenoma No. IU, hamster	8, 11
DMBA rat mammary cancer	14
S180 mouse sarcoma	6
Spontaneous C3H mouse mammary cancer	14, 15
Walker 256 rat carcinosarcoma	6, 16
Brown-Pearce rabbit epithelioma	6
T241 mouse sarcoma	11
V × 2 rabbit carcinoma	12, 13
Mast cell tumor, dog	10
Lymphoma, dog	10
Osteogenic sarcoma, dog	10
Human cancers, miscellaneous	4–7
Jensen rat sarcoma	19

COLD

Similar evidence for antitumor effects secondary to induced local or systemic hypothermia is not as abundant. In general, cold temperatures which permit resuscitation and do not involve tissue freezing have not resulted in in vivo regression of human and animal tumors, but exceptions have been reported. Table 2 lists these exceptions (18–21). It should be appreciated, however, that techniques for maintaining prolonged hypothermia in nonhibernating mammals with ready resuscitation had not been perfected experimentally at the time of these reports. Therefore, demonstrations of cold's inactivity against cancer may reflect only an insufficient duration of induced tumor hypothermia, particularly if pronounced or prolonged levels of cold are important.

EFFECTS OF TEMPERATURE CHANGE ON TISSUE GROWTH

There is good in vitro evidence that heat may alter cell-cycle times and growth rates in a variety of systems, normal and malignant. These thermal effects are not distributed equally throughout the cycle. Selawry et al showed that in HeLa and Hepatoma No. 2 cell lines,

TABLE 2.—Tumors in which cold produced regression in vivo

Tumor system	Result	Reference
Hamster methylcholanthrene sarcoma	No regression	18
Walker 256 rat carcinosarcoma	No regression	19, 20
Jensen rat sarcoma	Regression	20
Jobling-Flexner rat sarcoma	Regression	20
Human cancers, miscellaneous	Variable	21

mitosis is prolonged selectively when culture temperatures are elevated in vitro (22). Sisken and his associates showed that temperature affected all stages of the cell cycle (23). At a lower than optimal growth temperature, T + G_1 and metaphase were most prolonged. Metaphase was also most sensitive to the effects of an elevated temperature, being more prolonged than other cycle stages. These observations are of interest in that they show that progressing over the spectrum of temperature from 35 to 39 C, cell-cycle stages are prolonged, then shortened, and then lengthened once more as temperatures approach a level causing direct thermal injury. An implication of these data is that if these changes occur in vivo, then heat may act to stimulate tumor growth at one temperature, yet inhibit growth at another. In addition, in vitro data suggest that the duration of an induced temperature change is also important in effecting changes in generation time (24).

However, barring any such major induced changes in generation time in vivo, can the growth fraction of tumors be increased to therapeutic advantages by temperature elevation, ie, can the nonproliferating fraction of cells in a tumor be increased as a direct or indirect response to heat? The classic experiments of Huggins et al (25,26) suggested that heat can stimulate the nonproliferating stem-cell pool of normal hematopoietic tissue. They heated a rat's tail by implanting it in the abdomen, and thereby converted the nonproliferating yellow tail marrow to functional red marrow. Studies in the sand dollar suggest that the primary growth-stimulatory effect of temperature elevation is on the nondividing fertilized egg (27,28). Sufficient experimental evidence on this point related to cancer is not available, but such evidence is readily obtainable given current models and techniques.

Whether local or systemic temperature alterations can influence the therapeutic ratio of cytotoxic agents is pertinent to the question of the influence of temperature on tissue growth fraction. Does heat confer relative protection or sensitization to the cytotoxic effects of alkylating agents, antimetabolites, X-irradiation, or the alkaloids when one compares normal tissues to cancer? Heat is a recognized X-irradiation sensitizer, but the evidence indicates that heat will also sensitize normal tissues to X-irradiation (29). Chaffee and Musacchia noted a 40% decrease in tolerance to irradiation in heat-acclimatized animals (30). However, experimental tumors which are radioresistant in vivo can be cured when heat is used as adjunctive therapy (11). The optimal time-dose relationship between induced tumor hyperthermia and radiation is complex and as yet unresolved; it appears that the two must be employed simultaneously or at a very close interval. Unequivocal in vivo evidence that tumors heated above their normal basal temperatures take up a greater amount of a chemotherapeutic agent is lacking, although there is some suggestion that this is true (31,32). In tissue culture systems such an increased uptake is demonstrable (33). It is unlikely that heat can be considered in a two-dimensional fashion (more or no effect) regarding tumor growth in vivo. Effects ranging from tumor growth stimulation to inhibition and tumor death are demonstrable under differing heating conditions. This is perhaps best related to the kinetic studies by Sisken noted above.

There are considerable data that induced hypothermia affords protection to normal tissue from injury by cytotoxic agents when hypothermia is effected without extracorporeal circulatory assistance. Curiously, there have been little comparable data for malignant tissue. The effects of X-irradiation on cold tissues in vivo are markedly reduced as compared to normal tissues with regard to whole animal survival, marrow injury, and the mutagenic potential of irradiation (34–40). In profound hypothermia, more than twice the lethal dose of irradiation may be tolerated by mice (41). Studies concerned with cytotoxic drugs have been limited almost solely to mechlorethamine and other alkylating agents. Survival and bone marrow protection by cold are readily demonstrable and have permitted dosages to be employed which are several times larger than those found lethal in the normal intact animal (42–45). These observations at least imply that growth fraction and the mitotic cycle of tissue are altered by cold or heat. However, the necessary critical kinetic

analyses of tumors and normal tissue are lacking.

PHARMACOLOGY

In view of the major clinical commitment to the use of hypothermia in cardiac and neurosurgical procedures, there is a remarkable dearth of information on the influence of cold on drug metabolism and excretion. Understandably, perhaps, there is even less information on induced hyperthermic states. The few studies done show that systemic temperature changes influence both the excretion and metabolism of drugs by the various organs and that these may not occur in parallel at a given temperature. The work of Kalser et al with C^{14}-labeled atropine illustrates the dissimilarity of drug excretory responses of the liver and kidney to hypothermia; they showed that hepatic metabolism and excretion continued in the absence of renal handling of the drug (46). This is not surprising in view of the data on differential effects of hypothermia on organ blood flow. Blair has noted an exaggerated, depressed, or even normal response to different barbiturates and anesthetics during hypothermia (47). It is likely that similar observations would pertain to cancer chemotherapeutic agents since factors of detoxification, activation, and excretion are similarly important in such drugs' action. Thus, profound changes from normal in a drug's concentration in the plasma of a hypothermic man are to be anticipated at different temperatures. Popovic et al observed twice the blood levels of 5-fluorouracil during hypothermia in hamsters as they achieved at normal body temperatures, and they thought that an observed enhanced antitumor effect of the drug was due to the more prolonged time of effective drug concentration obtainable with low body temperatures (48).

An elevated systemic temperature is, of course, known to markedly influence cardiac output and heart rate. Most clinical studies have employed external heated environments or pyrogen-induced fevers to study the physiologic effects of heat in man. Grimby and Nilsson (49) and Koroxenidis et al (50) have shown that resting cardiac output and heart rate may approach a 50% increase with a 2-C rise in core temperature induced by pyrogens. Drug excretion studies in systemic hyperthermia have not been common. Results may vary depending on the techniques of inducing an elevated temperature. It seems likely that specific organ shunting of blood at elevated temperatures in man is, at least in part, dependent upon whether the heat source is environmental or central in origin. Recent data have confirmed that an impaired hepatic storage capacity of bromsulphalein (BSP) and an increased reflux from the liver to the plasma yield an abnormal BSP excretion in pyrogen-induced fever (51). Salicylamide plasma half-life and glucuronide conjugation are similarly reduced (52). Bischoff et al have developed a model for predicting pharmacologic kinetic data in man (53). Employing Bischoff's model and an approximate 50% increase in heart rate and cardiac output, one would anticipate that for a drug such as methotrexate, plasma concentrations after a single intravenous dose could be decreased during 2-C hyperthermia from 66% to 36% of normothermic levels with renal excretion rates increasing by 25% of normal (fig 1). In hypothermic states in the dog, where there are more physiologic data, a decrease in temperature from 38 to 30 C will increase the plasma concentration of methotrexate more than 2½-fold (fig 2).[6]

MECHANISMS OF TEMPERATURE EFFECTS

It could be considered self-serving to discuss a mechanism for the antitumor effects of heat or cold when there is at least a legacy of doubt that an effect exists. The radioprotective action of hypothermia has been explained most often by invoking tissue hypoxia which invariably accompanies current techniques of externally (non-perfusion-assisted) induced systemic lowering of temperature (54,55). Falls of tissue oxygen to 1–2 mg O_2 have been noted with profound hypothermia, and there is direct correlation between the radioprotective effect of cold and the level of induced tissue hypoxia. The influence of cold on the effects of anticancer alkylating agents could operate via a similar mechanism. Weiss makes

[6] Zaharko DS. Personal communication, 1973.

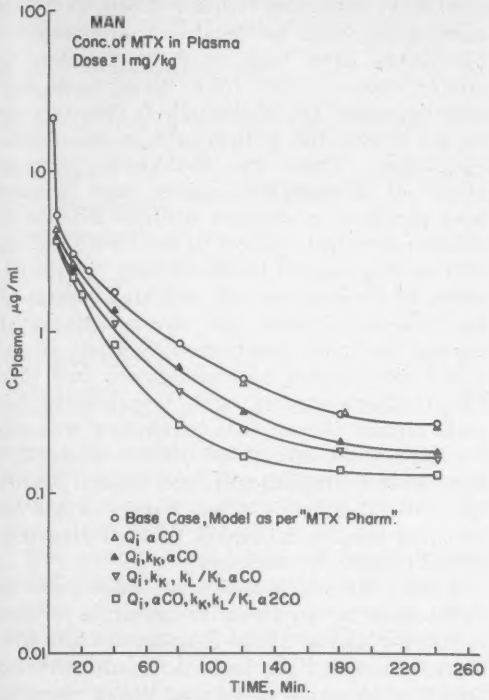

FIGURE 1.—Predicted temperature effects on plasma methotrexate concentration (in man). Base case is normal average 70-kg man with model parameters as per Bischoff et al (52); Q_i = blood flow to various body organs; CO = cardiac output; k_k = kidney clearance; k_L/K_L = liver clearance. Open circles represent plasma concentrations at 37 C body temperature. Other symbols represent plasma concentrations at 39–40 C body temperature with concentrations made for resulting changes in the noted physiologic parameters.

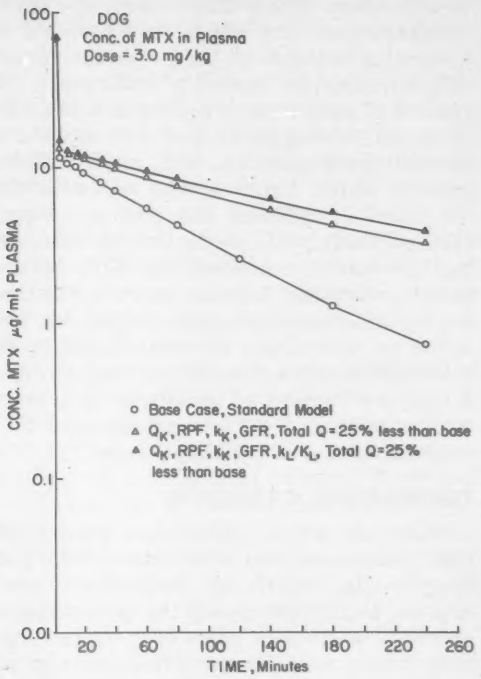

FIGURE 2.—Predicted temperature effects on plasma methotrexate concentration (in dog). Base case is normal average 17-kg dog with model parameters as per Bischoff et al (52); Q_K = blood flow to kidney; RPF = renal plasma flow; k_K = kidney clearance; GFR = glomerular filtration rate; Q = blood flow rate; k_L/K_L = liver clearance. Open circles represent plasma concentrations at 38 C body temperature. Other symbols represent plasma concentration at 30 C with corrections made for resulting changes in the noted physiologic parameters.

the interesting point that by rendering normal tissues anoxic by systemic hypothermia, one is gaining therapeutic advantage in radiation therapy (37). That is, since tumors are normally hypoxic, they sustain little additional change after hypothermia, with the net result that X-irradiation spares the normal, cold hypoxic tissue with no change in effect on the tumor. The idea has not yet been clinically tested. Harrison noted no change in the response of head and neck cancer to the alkylating agent ethylglycolid at hypothermic temperatures, but he did not do a controlled study at normal temperatures (55).

How heat may influence tumor regression is less clear when one is considering temperatures lower than those capable of coagulating tissue. An invariable accompaniment of hyperthermia is redistribution of blood flow. With local heating of an area there is evident surface hyperemia. If such hyperemia reflects a similar change in internal tumor blood flow at the arteriolar and capillary level, then alterations in pH, anaerobic metabolism, oxygenation, and penetration of host antitumor

factors may be readily brought forward for consideration. There have been no studies which compare the effects on tumors of the hyperemia induced by heat with the hyperemia inducible by vasoactive substances. The issue is of some interest in that if heat's effect is merely to exaggerate host defenses via increased tumor perfusion, and this is sufficient only to inhibit tumor growth partially, then drugs and/or radiation may play an adjuvant therapeutic role of greater utility than normal. It should be recalled that Crile believed that heat influenced tumor growth by enhancing the inflammatory response to cancer. Heat is known to produce marked changes in circulating leukocytes. It would be of some interest if the development of resistance in vivo of tumors to heat (11) was accompanied by a concomitant loss of local hyperemia.

TEMPERATURE GRADIENTS

There are ample clinical and animal data that demonstrate that with induced total body hypothermia, significant temperature gradients are established among the various organs and even in different parts of the same organ (56). These temperature differences may approach 10 C and are greatest when surface cooling methods are used, rather than blood perfusion techniques. Usually, gradients are largest during the induction phase of hypothermia and become less as temperature alteration is prolonged. However, in most clinical applications these differences are never totally dissipated. By themselves, such gradients can yield large differences in tissue oxygen tension and may also profoundly influence drug distribution. For example, Baldwin et al (57) noted that as the temperature dropped rapidly during experimental rapid cooling for neurosurgical studies, an apparent breakdown in the brain's exclusion of such substances as fluorescein, penicillin, and *d*-tubocurare was observed.

Although little has been published about the temperature patterns of primary or metastatic cancer in different parts of the body, the experience with mammary thermography indicates that at least one tumor is "hotter" than surrounding normal tissue (58). A tumor's temperature will probably vary at least in relation to its heat production (metabolic rate) and heat dissipation (tumor blood flow and surrounding tissue temperature). Angiographic techniques have been used most often to study tumors in vivo (59). While these may show increased vascular supply to tumors, they do not expose the critical area of the microcirculation. Even the well-known "tumor blush" of angiographic studies may indicate poor perfusion of tumors with persistence of radio-opaque dye relative to the "washout" of surrounding normal tissue. Indeed, central necrosis of tumors, low pH and high lactate in the interstitial fluid and venous effluent of tumors, and poor penetration of drugs all indicate a diminished microcirculatory flow (60–62). Gullino's studies with transplanted hepatic tumors showed that tumor flow was one twentieth that of normal liver tissue (63). Approached simplistically, one would predict that heat dissipation from tumors would be impaired relative to normal tissue during controlled induced hypothermia.

Several clinical investigators have tried to maintain or elevate tumor temperature relative to lowered normal tissue temperature (64–67). These attempts have been primarily directed toward a lessening of normal tissue response to cytotoxic cancer therapy rather than toward a modulation of tumor growth fraction. Usually, investigators have employed systemic hypothermia while maintaining the tumor area normothermic or hyperthermic during (usually) perfusion therapy. Unfortunately, these studies have not been controlled so as to permit comparison with perfusion therapy unaccompanied by temperature alteration, and data on the temperature response of the tumor to these techniques have usually not been generated. These clinical studies have almost uniformly employed alkylating agents. The clinical and animal studies using a "temperature gradient" approach are listed in table 3. Popovic et al, using their hamster cheek pouch system, had striking tumor regression with 5-fluorouracil at a normally ineffective dose when the drug was given to a hypothermic hamster with a normothermic tumor (48). Combination chemotherapy during induced systemic hypothermic states has not yet been used. One can only speculate on the potential

TABLE 3.—Anticancer drugs used in association with absolute or relative hyperthermia in animals or man

Drug	Reference
4,7,10,13-Tetraoxahexadecane, 1,2:15,16-diepoxy-	56, 58
2,5-Bis(1-aziridinyl)-3,6-bis(2-methoxyethoxyl)-*p*-benzoquinone (A139)	5
ThioTEPA	5
Cyclophosphamide	5
Mechlorethamine	5, 42–45
Melphalan	67
Triethylenemelamine	5
Actinomycin D	67
5-Fluorouracil	12, 48
Methotrexate	69

effects of modern drug therapy in Fay and Smith's patients of the 1930s who were maintained for up to 1 week at temperatures between 75 and 80 F (21–69).

The recent development of new extracorporeal membrane oxygenators (70) has permitted clinical perfusions of greater safety and more prolonged duration. They have been in use for as long as 2 weeks in various clinical situations requiring cardiopulmonary support. Such devices may permit the safe initiation of prolonged, controlled, systemic hypothermic states. If these techniques permit a desirable gradient between tumor and normal tissues to be maintained or augmented with body cooling, one should anticipate a quite useful adjuvant to the use of chemical or radiation therapy.

CONCLUSIONS

It is defensible to ask, given the large body of data needed to evaluate heat or cold as a potential adjuvant to cancer therapy, whether the clinical data to date encourage obtaining such experimental information with the permeations of cytotoxic agent, temperature, normal tissue, and tumor tissue. The experimental work necessary is substantial, particularly as temperature effects may be discontinuous on a given tissue and on effects on blood flow.

Clinicians have been particularly vigorous in pursuit of methods of inducing hyperthermia in man. They have encased the patient in heated wax, used heated breathed anesthetic gases, used hot blood perfusions to a limb or body part, and even employed the more traditional hot baths, heating coils, and microwave diathermy. Tumor tissue has been heated to 60 C clinically; blood perfusion techniques can take a limb to 41 C with safety for short periods. While these reports have repeatedly commented on the toxicity of such heating procedures, they also indicate, at times, dramatic tumor resolution. These therapies have not generally employed heat alone, but there are data in these clinical studies to support the observation that heat alone will cause regression of cancer (7).

While heat therapy may become of clinical importance in selected patients with accessible or superficial tumors, the approach remains trivial when one considers the more prevalent problem of disseminated cancer. Techniques and models are at hand to test the approach for systemic cancer with the view that existing temperature gradients may be manipulated toward therapeutic gain. Cold may play its greatest role in manipulating, with safety, temperature gradients between such disseminated tumors and normal tissues and provide, thereby, an expanded therapeutic ratio for the toxic effects of anticancer agents on normal tissue as compared with cancers.

It seems clear from this experience that the character of the current clinical data does not support development of a major experimental program. It would appear that what are now critically needed are well designed controlled studies where the effects of heat on tumors can be unequivocally evaluated. The complexity of such a trial is compounded by the disparate effects heat may have on tissues, but it is just such complexities which necessitate careful and precise control of future clinical efforts. Without such efforts it is unlikely that any broad clinical benefit of heat in cancer therapy will be rapidly appreciated.

LIST OF COMPOUNDS

Actinomycin D: NSC-3053

2,5-Bis(1-aziridinyl)-3,6-bis(2-methoxyethoxyl)-*p*-benzoquinone: NSC-17262; CAS reg. No. 800-24-8; A139

Cyclophosphamide: NSC-26271; CAS reg. No. 6055-19-2; 2*H*-1,3,2-oxazaphosphorine, 2-[bis(2-chloroethyl)amino]tetrahydro-, 2-oxide, monohydrate; Cytoxan

5-Fluorouracil: NSC-19893; CAS reg. No. 51-21-8; 5-FU

Mechlorethamine: NSC-762; CAS reg. No. 55-86-7; diethylamine, 2,2′-dichloro-*N*-methyl-, hydrochloride

Melphalan: NSC-8806; CAS reg. No. 3223-07-2; alanine, 3-[*p*-[bis(2-chloroethyl)amino]phenyl]-, monohydrochloride, L-

Methotrexate: NSC-740; CAS reg. No. 59-05-2; glutamic acid, *N*-[*p*-[[(2,4-diamino-6-pteridinyl)methyl]-methylamino]benzoyl]-

4,7,10,13-Tetraoxahexadecane, 1,2:15,16-diepoxy-: NSC-80439; CAS reg. No. 1954-28-5

ThioTEPA: NSC-6396; CAS reg. No. 52-24-4; phosphine sulfide, tris(1-aziridinyl)-

Triethylenemelamine: NSC-9706;CAS reg. No. 51-18-3; *s*-triazine, 2,4,6-tris(1-aziridinyl)-

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Comparative Studies on the Chemical Stability and Therapeutic Effectiveness of Water-Insoluble 5-[3,3-Bis(2-chloroethyl)-1-triazeno]imidazole-4-carboxamide (NSC-82196) and Its Water-Soluble Hydrochloride Against L1210 Mouse Leukemia [1,2,3]

Richard J. Woodman, Ira Kline, Ruth B. Field, and Miriam Gang [4]

SUMMARY

Treatment of L1210 leukemic mice with 5-[3,3-bis(2-chloroethyl)-1-triazeno]imidazole-4-carboxamide (NSC-82196) cured more than half the mice whether the agent was formulated and given as a suspension in an aqueous vehicle at pH 7.0 (susp 82196), or formulated and given as an aqueous solution of the hydrochloride at pH 2.0 (sol 82196). The antileukemic effectiveness of NSC-82196 in mice was retained at least three times longer after injection of susp 82196 than after injection of sol 82196. Stored at room temperature in the dark, a 50% conversion of NSC-82196 to a transformation product required 4 days for the suspension (pH 7) and approximately 4 hours for the solution (pH 2). The pure transformation product displayed a sharp dose response (eg, inactive and nontoxic at doses up to 350 mg/kg; acutely toxic at doses between 350 and 400 mg/kg). The antileukemic activity of the optimal therapeutic dose of freshly prepared sol 82196 (200 mg/kg) was sharply diminished when the dissolved drug was stored for more than 2 hours prior to injection. Injections of freshly prepared sol 82196 (400 mg/kg) killed 75% of the mice in 6.5 days, but after storage for 2 hours in the dark, injections of 400 mg/kg of this solution displayed marked antileukemic effectiveness. However, when sol 82196 was stored for 4 hours, 400 mg/kg was again acutely toxic to leukemic mice due to the increased content of the transformation product. The chemical instability of the hydrochloride salt of NSC-82196, when dissolved in water, emphasizes the need to protect the solutions from light and to use them immediately after reconstitution.

[Cancer Chemother Rep 57:383–391, 1973]

NSC-82196, a highly potent antitumor agent that is insoluble in water (1,2), displayed its optimal activity against murine L1210 leukemia when suspensions of the drug in water were injected intraperitoneally (ip), whereas when administered orally, it showed much less activity (3). Oral administration of this agent to patients resulted in very erratic absorption of the drug from the gut and very little anti-

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[2] Supported by contract PH43-68-1283 from the Division of Cancer Treatment (DCT), National Cancer Institute (NCI), National Institutes of Health, Department of Health, Education, and Welfare.

[3] NSC-82196: CAS reg. No. 5034-77-5. Supplied by Drug Research and Development (DR&D), DCT, NCI.

[4] Cancer Chemotherapy Department, Microbiological Associates, Inc., Bethesda, Md.

tumor activity (4). Consequently, a water-soluble parenteral formulation of NSC-82196 was developed by DR&D, DCT, NCI, by lyophilizing solutions of NSC-82196 in dilute HCl. The resulting microporous crystals of the hydrochloride salt, formulated for clinical use, were dissolved in 0.01N HCl at 20 mg/ml; these solutions have a pH of about 2.0. This product has recently undergone a clinical phase I trial and the results have been reported by Bagley et al (5).

Dry lyophilized NSC-82196 hydrochloride has shown no signs of chemical change after storage for 8 months at –10 C or below, but 90% of the acidic solutions of NSC-82196 incubated in the dark for 3 hours at 37 C are converted to the transformation product (6). The structure of this transformation product was elucidated by Abraham et al (7) as 1-(2-chloroethyl)-3-(5-carbamoylimidazol-4-yl)-Δ^2-1,2,3-triazolinium chloride (NSC-112970). When irradiated with long-wave ultraviolet light, NSC-82196 dissolved in 0.1 N HCl decomposes to 5-diazoimidazole-4-carboxamide which then cyclizes to 2-azahypoxanthine (6,8–10).

The chemical changes in acidic aqueous solutions of NSC-82196, and possible differences in route and schedule dependency and in vivo half-life of a water-soluble versus a water-insoluble formulation, prompted these comparative biologic studies using lifespan measurements of leukemic and nonleukemic mice as the main parameters in our test system.

MATERIALS AND METHODS

Formulation of Drugs

NSC-82196 (base) and its hydrochloride (lyophilized) were stored under desiccation at –15 C in brown glass bottles. All samples of the lyophilized hydrochloride were used within 2–5 months of synthesis.

Suspensions of NSC-82196 base (susp 82196) were prepared for injection by stirring appropriate amounts into steroid suspending vehicle[5] in brown glass bottles placed in crushed ice; the suspension was then injected as soon as possible.

The NSC-82196 hydrochloride solution (sol 82196) used in these experiments was prepared by injecting water into the capped vials containing the lyophilized material to obtain a final concentration of 20 mg/ml.[6] This concentration was given at 0.02 and 0.01 ml of drug solution/g of mouse body weight for doses of 400 and 200 mg/kg, respectively. Lower doses were prepared by further dilution with water using 0.01 ml of drug solution/g of body weight. Sol 82196 was injected within 5 minutes of preparation, except in drug-aging experiments which are noted specifically.

The transformation product (NSC-112970) was dissolved in 0.1N NaCl and injected ip at pH 4.4.

Evaluation of Therapeutic Effectiveness and Toxicity of Drugs In Vivo

Therapeutic effectiveness and toxicity of sol 82196 and susp 82196 under varying conditions were evaluated in BDF_1 mice (or CDF_1 mice where indicated). For evaluation in leukemic mice, 10^5 ascitic L1210 leukemic cells were injected ip (Day 0) and treatment began on Days 1 or 5. Details of these procedures have been reported in a previous paper (3).

Estimation of Residual Antileukemic Activity in Mice 15 or 30 Minutes After an Injection of Sol 82196 or Susp 82196

A shortened form of the bioassay method of Kline et al (11,12) was used to estimate the relative residual antileukemic activity after an injection of sol 82196 as compared with susp 82196. This bioassay method indirectly measures the decrease of antileukemic drug levels based on survival times of mice challenged with L1210 leukemic cells at increasing time intervals after drug administration. Dose-response curves for simultaneous cell and drug injection were obtained by injecting 10^5 L1210 cells ip followed within 5 seconds by graded doses of susp 82196 or sol 82196 given ip, orally, or intravenously (iv) to establish maxi-

[5] One ml of steroid suspending vehicle contained 9 mg of NaCl, 5 mg of sodium carboxymethylcellulose, 0.004 ml of polysorbate-80, 0.0009 ml of benzyl alcohol, and water to volume. Suspensions of NSC-82196 in the vehicle had pH values of about 7.0.

[6] Sol 82196 samples used in these experiments were dissolved at concentrations of 20 mg/ml of distilled water rather than 0.01N HCl, which yielded solutions with pH values of 1.8–2.2.

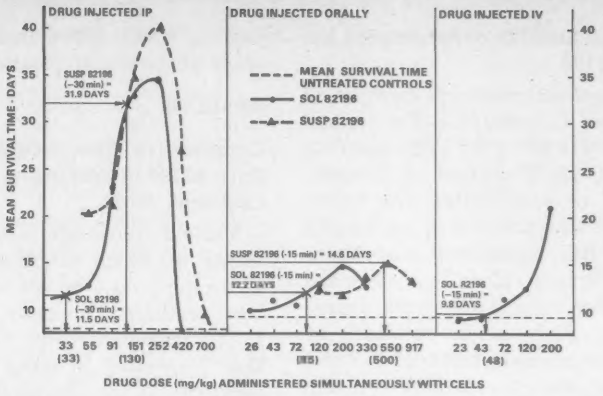
DRUG INJECTED IP
DRUG INJECTED ORALLY
DRUG INJECTED IV
MEAN SURVIVAL TIME - DAYS
MEAN SURVIVAL TIME UNTREATED CONTROLS
SOL 82196
SUSP 82196
SUSP 82196 (-30 min) = 31.9 DAYS
SOL 82196 (-30 min) = 11.5 DAYS
SUSP 82196 (-15 min) = 14.6 DAYS
SOL 82196 (-15 min) = 12.2 DAYS
SOL 82196 (-15 min) = 9.8 DAYS
33 55 91 151 252 420 700
(33) (130)
26 43 72 120 200 330 550 917
(500)
23 43 72 120 200
(48)
DRUG DOSE (mg/kg) ADMINISTERED SIMULTANEOUSLY WITH CELLS

FIGURE 1.—Dose-response curves of mice injected ip with 10^5 L1210 leukemic cells followed within 5 seconds by sol 82196 or susp 82196 at dose levels and via routes shown. Mean survival time of mice injected ip with drug 30 minutes before injection of leukemic cells and orally or iv 15 minutes before injection of leukemic cells read off dose-response curves to give "residual" drug dose. These residual doses are shown in parentheses under the administered dose scales.

mal antileukemic activity at zero time interval. These curves, shown in figure 1, were used to translate mean survival times into antileukemic drug dose levels in the host at the time of tumor cell injection. Parallel groups of mice were injected ip with graded doses of sol 82196 or susp 82196 at time intervals ranging from 15 minutes to 24 hours before ip injection of 10^5 L1210 cells. The maximal mean survival time of these mice was then extrapolated from the dose-response curve (fig 1) to obtain the amount of NSC-82196 injected simultaneously with L1210 cells that would have produced the same mean survival time. This amount was considered to be the estimated retained dose of NSC-82196 and was expressed as a percentage of the administered dose of sol 82196 or susp 82196 (table 1).

TABLE 1.—Relative decrease of antileukemic activity after injection of sol 82196 compared with susp 82196 injected by various routes

Drug	Route of injection	MST (days) at optimal dose (drugs and cells injected simultaneously)	Optimal dose (mg/kg)	Time interval* (min)	Mean survival time after delayed cell injection† (days)	Estimated residual dose‡ (mg/kg)	Residual dose§ (%)
Experiment A¶							
Sol 82196	ip	34.5	252	30	11.5	33	13
Susp 82196	ip	40.0	252	30	31.9	130	52
Experiment B¶							
Sol 82196	oral	14.4	200	15	12.2	85	42
Susp 82196	oral	14.8	550	15	14.6	500	91
Sol 82196	iv	21.2	200	15	9.8	<48	<24

*Time between injection of drug and ip injection of 10^5 L1210 leukemic cells.

†Mean survival time of mice injected with L1210 cells at the corresponding time interval after drug injection.

‡Obtained by extrapolating mean survival time values in preceding column from the standard dose-response curves (fig 1).

§Values of estimated residual dose expressed as percent of administered dose.

¶Mean survival time of untreated controls: experiment A = 8.3 days; experiment B = 9.5 days.

Measurement of the Stability of Formulated Sol 82196 and Susp 82196

Measurement by direct chemical analysis of stored sol 82196 and susp 82196.—The method of Loo and Stasswender (10) was used to determine the NSC-82196 content of formulations of sol 82196 or susp 82196. The transformation product was assayed in an aliquot dissolved in 0.1N HCl, measuring absorbance at 235 nm and referring values to a standard curve of absorbance values for pure transformation product. Since acidic solutions of NSC-82196 also have an absorbance at 235-nm, correction was made for this component in the sample by subtracting the 235-nm absorbance value due to the known amount of NSC-82196 in the sample from the total 235-nm absorbance value.

Measurement by evaluation of survival of mice after injection of stored sol 82196 and susp 82196.—Mice were injected ip with 10^5 L1210 leukemic cells (3) at time zero and received injections 24 hours later with either sol 82196 or susp 82196 that had been stored in the dark at room temperature for varying periods up to 7 days. Nonleukemic mice similarly received injections with stored samples of sol 82196. The mice were observed for survival for a period of 60 days in order to correlate survival times with chemical analyses. Immediately after injection of the stored drug, aliquots were removed for chemical analysis of NSC-82196 and spectrophotometric assay of the transformation product.

RESULTS

Comparative Effectiveness of Sol 82196 and Susp 82196 in Prolonging the Lifespan of L1210 Leukemic Mice

Table 2 shows the results of several experiments in which the therapeutic effectiveness of a single dose of sol 82196 and susp 82196 was compared in early or advanced ascitic L1210 leukemia. Both formulations given on Day 1 resulted in more than 50% long-term survivors at the 200-mg/kg dose. Compared to susp 82196, sol 82196 given on Day 5 was less effective in one experiment (2*a*) and equally effective in another (2*b*) in prolonging lifespan, but both formulations resulted in less than 50% long-term survivors with this delayed treatment. It is interesting to observe that mice with advanced L1210 leukemia will tolerate higher doses of sol 82196 (400 mg/kg) than mice treated at an earlier stage of disease.

With respect to the schedule-dependency of sol 82196 against early L1210 leukemia, the data of table 3 show that all treatment schedules except daily on Days 1–9 gave 50% or more 60-day survivors. Of all the treatment schedules giving more than 50% long-term survivors, the single or multiple treatments on Day 1 only gave the best 60-day survival rate (seven of

TABLE 2.—Therapeutic effectiveness of sol 82196 and susp 82196 against early and advanced L1210 leukemia

Experiment	Drug*	Day of treatment	%ILS (long-term survivors)†				
			800 mg/kg	400 mg/kg	200 mg/kg	100 mg/kg	50 mg/kg
1*a*§	Sol 82196	1	Toxic (0/8)‡	Toxic (1/8)	>567 (7/8)	67 (1/8)	
1*b*	Sol 82196	1		Toxic (0/8)	>567 (5/8)	61 (0/8)	
	Susp 82196			184 (2/8)	233 (2/8)	84 (1/8)	
1*c*	Sol 82196	1		Toxic (3/8)	>361 (8/8)	11 (3/8)	0 (0/8)
	Susp 82196			361 (7/8)	>361 (7/8)	26 (2/8)	0 (0/8)
2*a*	Sol 82196	5	Toxic (0/8)	89 (1/8)	61 (0/8)	22 (0/8)	
	Susp 82196		317 (3/8)	278 (0/8)	111 (0/8)	44 (0/8)	
2*b*	Sol 82196	5		106 (2/8)	78 (0/8)	11 (0/8)	6 (0/8)
	Susp 82196		17 (0/8)	111 (0/8)	111 (1/8)	55 (0/8)	

*Drugs injected once ip on Days 1 or 5 after ip injection of 10^5 ascitic L1210 leukemic cells.

†%ILS = percent increase in lifespan. Test terminated on Day 60 (experiments 1*a* and 1*b*), Day 55 (experiment 1*c*), Day 54 (experiment 2*a*), Day 40 (experiment 2*b*).

‡More than 4/8 mice died by Day 9.

§MST was 13.0 days for experiment 1*c* and 9.0 days for all other experiments.

TABLE 3.—Treatment schedule dependency of the antileukemic activity of sol 82196 against early L1210 leukemia*

Sol 82196 (mg/kg/ day, ip)	Day 1 only		q3h, Day 1†		Daily, Days 1–9		Days 1,5, and 9		Days 1 and 9	
	MST‡	%ILS§	MST	%ILS	MST	%ILS	MST	%ILS	MST	%ILS
800	2	0	18 (3)	100						
400	8 (1)	0	>60 (8)	>567			9	0	9	0
200	>60 (7)	>567	>60 (5)	>567	9	0	>60 (5)	>567	>60 (5)	>567
100	15 (1)	67	13	44	23 (1)	155	25 (1)	178	23	155
50					20	122				
25					18.5	106				

*10^5 Ascitic L1210 cells injected ip, Day 0. MST of untreated controls = 9.0 days.
†q3h = Day's dose divided into 8 injections, each given 3 hours apart.
‡Figures in parentheses = No. of 60-day survivors, 8 mice per group.
§%ILS = percent increase in lifespan.

eight and eight of eight animals surviving). These results were similar to the schedule-dependency data for susp 82196 (3).

Table 4 shows that response of early L1210 leukemia to sol 82196 was influenced by the route of administration. In this particular experiment, sol 82196 injected subcutaneously gave little increase in the median survival time (MST), although there were two 60-day survivors at the optimal dose of 400 mg/kg. Orally administered sol 82196 (MST, 30 days) was much less effective than ip administered drug (MST, >60 days). A similar difference between the two injection routes was found in earlier studies with susp 82196 (3). Iv and ip injections of sol 82196 gave five of ten and eight of ten 60-day survivors, respectively. For comparison, the therapeutic effect of ip treatment with susp 82196 in the same test was similar to that of ip treatment with sol 82196.

Estimation of the Relative Residual Antileukemic Activity After Injection of Sol 82196 Compared With Susp 82196

The data of table 1 show that 30 minutes after ip injection of sol 82196, 14% of maximal activity at the optimal dose (252 mg/kg) is retained as compared with 52% for susp 82196.

After oral injection, 42% of the maximal activity of an optimal dose of sol 82196 (200 mg/kg) is retained at 15 minutes as compared with 91% of the maximal activity of susp 82196 at the optimal dose of 550 mg/kg.

These two sets of data clearly show that residual antileukemic activity decreased more rapidly after administration of sol 82196 than after administration of susp 82196. Fifteen

TABLE 4.—Injection route dependency of the antileukemic activity of sol 82196 against early L1210 leukemia *

Drug†	Route of injection	Optimal dose (mg/kg)	%ILS ‡	60-day survivors/ total treated
Sol 82196	oral	400	30	3/10
	iv	200	>188	5/10
	sc	400	7	2/10
	ip	200	>361	8/10
Susp 82196	ip	200, 400	>361	7/10, 7/10

*10^5 Ascitic L1210 cells injected ip, Day 0.
†Sol 82196 and susp 82196 injected once, Day 1.
‡%ILS = percent increase in lifespan. MST of untreated controls = 9.0 days.

minutes after iv injection of sol 82196 there was scarcely any increase in lifespan in mice challenged with 10^5 L1210 cells as compared with controls that were injected with L1210 cells only. This suggests that residual antileukemic activity had virtually disappeared from mice injected iv with sol 82196 in less than 15 minutes.

Sol 82196 and susp 82196 injected simultaneously, ip or orally, gave similar maximal survival responses (MST = 34.5 versus 40.0 days, or 14.4 versus 14.8 days, respectively). By contrast, the duration of antileukemic activity of the two formulations at their optimal doses was very different. The results shown in table 1 also confirm the superiority in therapeutic effectiveness of the ip injection route over the oral route (table 4).

Measurement of the Chemical Stability of Preparations of Sol 82196 and Susp 82196

By chemical methods.—Figure 2 shows the decreasing amount of NSC-82196 that can be recovered from sol 82196 at increasing time periods up to 48 hours after addition of the vehicle. At a concentration of 10 mg of sol 82196/ml, more than half the NSC-82196 content had disappeared by the time the drug had been stored for 4 hours at room temperature in the dark. Simultaneously, there was an increase in transformation product. By contrast, the rate of diminution of NSC-82196 in samples of susp 82196 was much slower. Table 5 shows that for the 19.2-mg susp 82196 sample, the NSC-82196 diminished to one half its original level at 3.5 days, and as the concentration of drug was increased, the decrease in NSC-82196 became slower. At the lowest concentration (1.0 mg/ml) it took only 6 hours for the NSC-82196 to be reduced by one half, which is almost as rapid as for the 10-mg/ml preparation of sol 82196. Hence, the rate at which susp 82196 dissolves may limit its rate of conversion to transformation product, and these rates possibly increase as the pH falls toward 5.5. The pH of susp 82196 preparation at all concentrations fell from an initial value of 7.0 to approximately 5.5 by the end of the observation period.

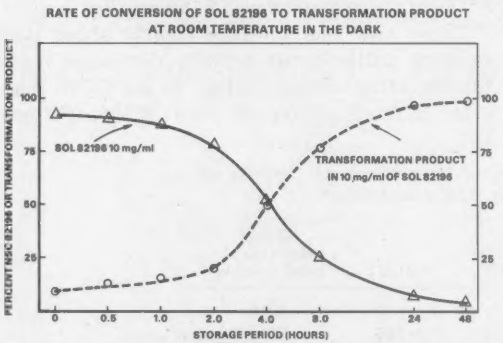

FIGURE 2.—Sol 82196 dissolved at a concentration of 10 mg/ml of water to give solutions with pH = 2.0 at time zero. After storage as shown, samples were removed and analyzed for NSC-82196 and transformation product as described in text (Methods). Concentrations of these components are expressed as a percentage (molar basis) of the drug concentration at time zero.

By in vivo methods.—Table 6 shows the survival of leukemic mice after treatment at three dose levels of susp 82196 or sol 82196 stored in the dark for varying periods. Data for nonleukemic mice injected with sol 82196 stored for varying periods are also included.

For mice treated with susp 82196, table 6 shows that 392 mg/kg was the optimal dose for

TABLE 5.—Rate of conversion of various concentrations of susp 82196 in vitro

Formulated concentration of susp 82196* (mg/ml)	Time for formulated concentration to decrease by 50%† (days)
80.0	9.4
56.0	8.8
39.2	6.9
27.4	5.3
19.2	3.5
1.0	0.25

*NSC-82196 suspended at these concentrations in steroid suspending vehicle and stored in brown glass bottles at pH 7.0 at room temperature for up to 15 days. Residual NSC-82196 content assayed after various time intervals by the method of Loo and Stasswender (10).

†Drug sampled at 0, 4, and 8 hours, 1, 2, 3, 4, 7, 8, 9, 10, 11, and 15 days after formulation and curves for percent residual NSC-82196 plotted. Time taken to reach 50% NSC-82196 content of each formulated concentration derived from these curves.

TABLE 6.—Antileukemic activity and toxicity of formulated sol 82196 after storage in the dark at room temperature

Duration of storage	Dose level of sol 82196 injected into leukemic mice *						Dose level of sol 82196 injected into normal mice			
	400 mg/kg		200 mg/kg		100 mg/kg		400 mg/kg		200 mg/kg	100 mg/kg
	MST (days)	Survival †	MST (days)	Survival	MST (days)	Survival	MST (days)	Survival	Survival	Survival
<15 seconds	6.5	2/8	>40	6/8	14.5	5/8	6.5	0/8	7/8	8/8
0.5 hours	8.5	1/8	>40	6/8	13.0	0/8				
1.0 hours	6.5	0/8	>40	5/8	14.0	0/8				
2.0 hours	>40.0	6/8	>40	5/8	13.0	0/8	>60.0	6/8	8/8	8/8
4.0 hours	1.0‡	3/8	15.0	1/8	12.0	0/8				
8.0 hours	1.5‡	0/8	13.5	0/8	10.5	0/8				
1.0 day	1.0‡	0/8	10.0	0/8	9.0	0/8	1.0‡	0/8	8/8	7/8
2.0 days	1.0‡	0/8	10.0	0/8	9.0	0/8	1.0‡	0/8	8/8	8/8

*pH of freshly formulated sol 82196 = 2.1; 10^5 ascitic L1210 cells injected on Day 0; all injections of sol 82196 given once on Day 1.

†Survival = No. of survivors of 8 treated on Day 60 (normal mice) or Day 40 (leukemic mice). MST of untreated leukemic mice = 9.0 days.

‡More than 4/8 mice died within 4 hours of injection.

the freshly formulated drug (MST, 43.5 days). After storage for 8 hours, the same dose of susp 82196 gave an MST of more than 60 days (six 60-day survivors of eight treated), and after storage for 7 days the MST resulting from this dose decreased to 21.5 days. By contrast, the maximal MST (>40 days) obtained after injection of 200 mg/kg of freshly prepared sol 82196 was reduced to 15 days if this same dose was injected after storage for 4 hours.

The survival responses in leukemic mice were in agreement with chemical analyses of stored NSC-82196 formulations in which sol 82196 was shown to break down into toxic and therapeutically inactive transformation product much more rapidly than susp 82196. These survival responses also confirm the data of Dresback and Gallelli (8) on the chemical stability of sol 82196 in vitro.

The data of table 6 also describe the toxicity of stored sol 82196 after injection into nonleukemic mice. Freshly formulated sol 82196 at a dose level of 400 mg/kg was lethal to all mice (MST, 6–5 days). The same dose of sol 82196 stored for 1–2 days was lethal to all the mice within 4 hours of injection. From the analytical data of figure 2, the toxicity of 400 mg/kg of sol 82196 was probably due to two components. The freshly formulated drug killed all the mice due to its NSC-82196 content, but the drug stored for 1–2 days probably killed them because of its greater content of transformation product. Presumably, the toxicity of these two components was not additive, since the 400-mg/kg dose of drug stored for 2 hours before injection which contained both NSC-82196 and transformation product (*see* fig 2) killed only two of eight mice. Figure 2 suggests that under these conditions the mice received about 300 mg/kg of NSC-82196 and 100 mg of transformation product.

The observation that sol 82196 stored for more than 2 hours was acutely toxic to leukemic mice after a single dose of 400 mg/kg was further investigated by injecting nonleukemic mice with closely spaced doses of transformation product. The data in table 7 show that there was a sharp increase in acute toxicity from 25% to 75% mortality in only one increment from 369 to 410 mg/kg. The complete conversion of the 400-mg/kg dose of sol 82196,

TABLE 7.—Toxicity of transformation product (NSC-112970) after 1 ip injection into normal mice*

Transformation product dose (mg/kg)	No. of deaths within 1 hour of injection among 8 mice †
450	7
410	6
369	2
333	3
300	1
270	0

*CDF, male mice weighing 20–25 g were used.
†All mice surviving 1 hour after injection of NSC-112970 were still alive 10 days later.

which was the optimal therapeutic dose for mice with advanced L1210 leukemia (table 2), would therefore result in lethal toxicity to at least half the mice. In other species, the conversion of therapeutic doses of sol 82196 to transformation product prior to injection might well expose the host to toxicity from this substance.

DISCUSSION

These studies were designed to investigate three main aspects of the water-soluble formulation of NSC-82196. These were the comparative therapeutic effectiveness of sol 82196 versus susp 82196, the practicality of using sol 82196 parenterally (by the iv route), and the chemical stability of sol 82196 in vitro and in vivo.

The results in table 2 show that sol 82196 and susp 82196 give a similar therapeutic effect against early or advanced L1210 leukemia. Treatment schedule-dependency for the two formulations against early L1210 leukemia is also similar. This is an interesting observation in view of the large differences between the two formulations in duration of circulating antileukemic drug (table 1).

Since the main reason for converting NSC-82196 to a water-soluble form was to have an iv formulation, it is important to note that sol 82196 injected iv retained a high level of therapeutic activity (five 60-day survivors of ten treated, table 4). The other advantages of an iv water-soluble form include accurate dispensation from a syringe and rapid access of the drug to the tissues. In addition, oral injection of NSC-82196 is less effective against L1210 leukemia than ip injection (3), and NSC-82196 shows very little activity when given orally to patients (4).

These studies did, however, reveal certain properties of sol 82196 that call for precautions in the storage, handling, and administration of this formulation. In our experience with the samples of sol 82196 used in these experiments, storage of the lyophilized drug at temperatures higher than –15 C resulted in partial conversion to transformation product after several months. This process was greatly accelerated after the addition of the vehicle and was 50% complete after 4 hours at room temperature at the low pH of 2.0 of the formulation (2) (fig 2). Undue delay in injection of a calculated therapeutic dose of the drug might then result in a loss of antitumor activity and the inadvertent administration of a potentially toxic dose of transformation product.

The acidity of the solutions of sol 82196 (< pH 2.0) suggests undesirable side effects, such as necrosis at the injection site, precipitation of serum protein, and acidosis. Preliminary experiments in our laboratory showed that mice will tolerate an iv injection of not more than 0.2 ml (200 mg/kg) of sol 82196 at pH 2. The addition of 0.2 ml of sol 82196 (20 mg/ml) to 1.0 ml of heparinized mouse plasma, buffered with sodium bicarbonate/CO_2 at room temperature, caused the pH to fall from 7.4 to 6.2 with simultaneous formation of an amorphous precipitate. Since iv injection of a 200-mg/kg dose of sol 82196 was not lethally toxic, such a large fall in pH could not occur in vivo. Even so, this suggests that mice surviving a 200-mg/kg iv dose are exposed to a severe acid load. Acidosis produces several physiologic effects, including ion imbalance and impaired renal function that could, in turn, influence the excretion rate of active metabolites of NSC-82196. Such an event might provide a possible explanation for the rapid decrease in retention of antileukemic activity after the injection of sol 82196 as described in table 1. A more likely explanation, however, of the differences in retention of antileukemic activity is the slow rate at which susp 82196 dissolves and diffuses into the blood stream compared with the likelihood of rapid vascular dissemination of water-soluble sol 82196.

The availability of a water-soluble form of NSC-82196 facilitates accurate iv injection of this drug. However, our animal studies, like those of other investigators (6,8), emphasize the potential instability of sol 82196 and the danger of inadvertent administration of toxic conversion products with concomitant loss of antitumor activity. Our observation that acidic solutions of sol 82196 do not revert to an insoluble form when added to *whole* mouse blood, suggests the interesting possibility of water-insoluble drugs being conveyed through the vascular system dispersed on the surfaces of blood cells or other particulate matter.

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Pyrogenic Properties of Bleomycin (NSC-125066) [1,2]

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SUMMARY

The antitumor agent bleomycin was shown to be pyrogenic when injected intravenously (iv) into rabbits. During the height of bleomycin-induced fever a circulating, rapid-acting pyrogen was transferable to other rabbits. In addition, rabbit and human white blood cells incubated overnight with bleomycin released a similar rapid-acting pyrogen. Significant pyrogenic tolerance did not develop in rabbits receiving daily iv injections of the drug. No cross tolerance to bacterial endotoxin was demonstrated. These experiments have shown that the chills, fever, and occasional shock-like state produced in man by bleomycin are not caused by contaminating bacterial endotoxin. It is suggested that the drug produces fever by liberating an endogenous pyrogen from the host's cells.

[Cancer Chemother Rep 57:393–398, 1973]

Bleomycin is a new antitumor agent used primarily to treat squamous cell carcinoma of the skin, urogenital organs, head, neck, and esophagus; it is used to a lesser extent to treat melanoma, bronchogenic carcinoma, and lymphoma (1,2). In man and animals, it is taken up primarily by the squamous epithelium (3), and it appears to interfere with DNA synthesis (4,5). The drug has no significant bone marrow toxicity (2,6), although other side effects such as pulmonary fibrosis may be pronounced (2,7,8).

Fever is a major toxic reaction in 25% of the patients treated with bleomycin (2,9), but in patients with lymphoma the incidence of fever increases to nearly 90% (2,10–14). Of particular interest, however, is the ability of the drug to cause acute febrile reactions (often associated with chills and hypotension and lasting from 8 to 18 hours) within 2 hours of the administration of the initial dose (10–14). Review of the clinical histories of many patients with lymphoma who experienced acute responses suggested to us that there are many similarities between the reaction to bleomycin and the reactions seen after the administration of bacterial endotoxin. The following experiments were designed to investigate the pyrogenic properties of bleomycin.

MATERIALS AND METHODS

Rabbits.—New Zealand albino rabbits of both sexes were used. The animals were bred at NIH and weighed approximately 2 kg each. The rabbits were housed and tested in an air-conditioned room.

Temperature recordings.—Rectal temperatures (degrees centigrade) were obtained with thermistor probes connected to an automatic recording device (Yellow Springs Instrument

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[2] Bleomycin (lot No. 71L481) was supplied by Bristol Laboratories, Syracuse, NY, through Drug Research and Development, Division of Cancer Treatment, National Cancer Institute.

[3] Laboratory of Clinical Investigation, National Institute of Allergy and Infectious Diseases, National Institutes of Health (NIH), Bethesda, Md.

[4] The authors thank Dr. Ronald Elin for performing the limulus amebocyte lysate tests.

Co., Yellow Springs, Ohio). Recordings were made every 12 minutes. Rabbits were trained in wooden stocks on the day prior to testing. During experiments, a baseline which did not vary more than 0.2 C in 1 hour before the injections was established. Fevers were plotted on 1 × 1-inch graph paper where 1 hour and 1 degree each equaled 1 inch. The area in square centimeters under a 5-hour fever curve was measured with a compensating polar planimeter (Keuffel and Esser, Hoboken, NJ) and designated as the fever index (15).

Bleomycin.—Bleomycin was supplied in 15-mg ampuls. Just prior to use, the drug was dissolved in 0.9% pyrogen-free saline. All injections were given intravenously (iv) through the lateral ear vein. All solutions and glassware were sterile and pyrogen free.

Endotoxin.—A Boivin preparation of *Escherichia coli* 0127 endotoxin (Difco) was used in all experiments.

Tolerance to endotoxin.—Rabbits were made tolerant to endotoxin by six daily iv injections of 3 μg of *E. coli* endotoxin.

White blood cell (wbc) counts.—Total and differential peripheral leukocyte counts were done during bleomycin-induced fever. A new small incision was made in a lateral ear vein to obtain blood for each count. Control samples were taken 1 hour before and immediately before the drug was injected. Total wbc counts were determined in an electronic particle counter (Coulter Electronics, Hialeah, Fla), and smears were made and stained with Wright's stain. All differential counts were made on 100 cells.

Transfer of circulating pyrogen.—Rabbits given bleomycin were exsanguinated 5 hours after injection of the drug and the plasma was immediately separated from heparinized blood. Plasma was frozen at –10 C and thawed just prior to use. All plasma was sterile.

RESULTS

Pyrogenic properties of bleomycin.—Groups of rabbits were given increasing doses of bleomycin (0.08, 0.8, 4.0, and 8.0 mg/kg), and febrile responses were plotted. The dose of 0.08 mg/kg did not produce fever, but a dose ten times this amount produced small, monophasic fevers after a latent period of 2 hours. Larger doses produced correspondingly higher fevers; these fevers were characterized by a shorter latent period and more prolonged monophasic elevation (fig 1). A dose-response relationship was established (fig 2), and it was possible to choose a dose that would allow us to make quantitative comparisons in later experiments.

Bleomycin-induced endogenous pyrogen.—The mechanism by which pyrogenic substances cause fever is thought to be mediated through a small protein called endogenous pyrogen. This protein after its release from phagocytic cells is thought to stimulate the thermoregulatory center of the hypothalamus (16). This transferable pyrogen can be demonstrated in the circulation during a variety of experimental fevers in rabbits including endotoxin fever (17).

To ascertain whether bleomycin induces production of endogenous pyrogen, rabbits were given 8 mg/kg of bleomycin and exsanguinated during the height of fever (fig 3). Recipient rabbits received injections of 10 ml/kg of febrile plasma. All recipients had brief, monophasic fevers with a latent period of 15–20 minutes; this is characteristic of fever induced by endogenous pyrogen (fig 3). In additional experiments, heparinized rabbit or human blood was sedimented with dextran, and the leukocytes were washed and suspended in Hank's Balanced Salt solution. Bleomycin

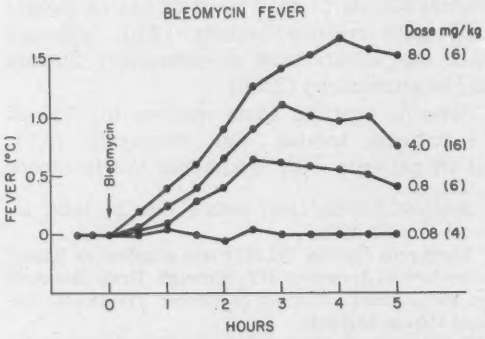

FIGURE 1.—Mean febrile responses of rabbits treated with various doses of bleomycin given iv at 0 hours. The numbers in parentheses indicate the number of rabbits in each group.

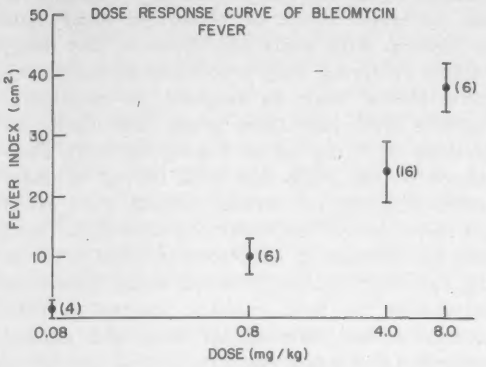

FIGURE 2.—Mean 5-hour fever indices (± standard error) recorded from rabbits treated iv with increasing doses of bleomycin. The numbers in parentheses indicate the number of rabbits in each group.

(2 mg/50 × 10^6 leukocytes) was added to cell suspensions and incubated at 37 C for 18 hours. Control incubations were made without the drug. After centrifugation at 3000 rpm for 30 minutes, supernatant fluids were injected into rabbits at a dose equivalent to 50 × 10^6 leukocytes. Whereas control supernatant fluids did not produce fever, supernatant fluids from bleomycin incubations produced rapid-onset, monophasic fevers similar to those demonstrated from febrile plasma. However, these fevers were occasionally prolonged up to 4 hours; this may indicate the presence of the drug in the supernatant fluids.

Leukocyte counts during bleomycin-induced fever.—Both the febrile course and the change in circulating leukocytes were plotted (fig 4). After 5 hours the total count remained low, but after 24 hours the counts had returned to normal. There was no significant change in the percentage of polymorphonuclear leukocytes, monocytes, and lymphocytes despite a marked reduction in the total count (fig 4).

Effect of daily iv injections of bleomycin.—Daily iv treatment with bacterial endotoxin results in a pyrogenic refractoriness which is called endotoxin tolerance. Such states of pyrogenic refractoriness have also been observed in other experimental fevers (18,19). In an attempt to demonstrate tolerance to bleomycin, rabbits were given five daily injections of the drug, and their temperatures were recorded on the first and fifth day. On the fifth day slight tolerance was demonstrated (fig 5). Although the latent period was prolonged to 2 hours on the fifth day (in comparison with a

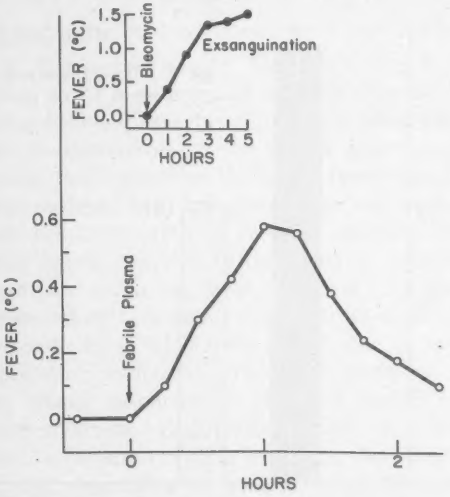

FIGURE 3.—Upper graph: mean fever above baseline in five rabbits treated with 8 mg/kg of bleomycin (arrow) and exsanguinated after 5 hours. Lower graph: mean fever above baseline in eight rabbits given 10 ml/kg of febrile plasma (arrow).

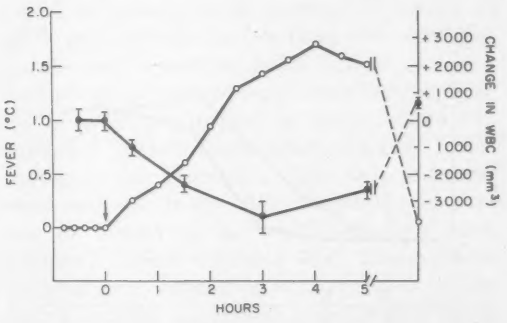

FIGURE 4.—Mean fever (○———○) above baseline of five rabbits treated with 8 mg/kg of bleomycin at 0 hours. The mean change in the total wbc counts (± standard error) of the same rabbits (●———●) is shown on the right ordinate. The final points indicate values at 24 hours.

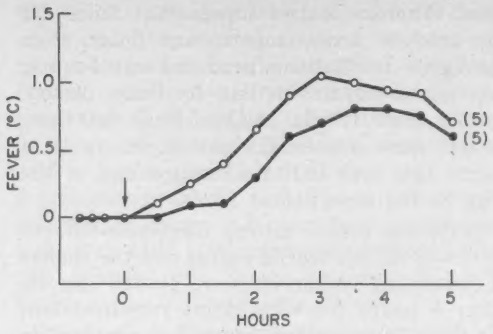

FIGURE 5.—Mean febrile responses of five rabbits on the first day (○———○) after treatment with 4 mg/kg of bleomycin and on the fifth day (●———●) of five daily injections given at 0 hours.

latent period of 45–60 minutes after the initial dose), the fever indices did not change significantly ($P > 0.2$, "t" test).

Bleomycin fever in endotoxin-tolerant rabbits.—Endotoxin-tolerant rabbits are capable of having fever from other pyrogens (18,19). Rabbits made tolerant to endotoxin were challenged on the sixth day with 4 mg/kg of bleomycin. When compared with nontolerant rabbits, endotoxin-tolerant rabbits had no reduction in bleomycin-induced fever (fig 6). This lack of cross tolerance between endotoxin and bleomycin suggests that the pyrogenicity of the drug is not due to contaminating endotoxin.

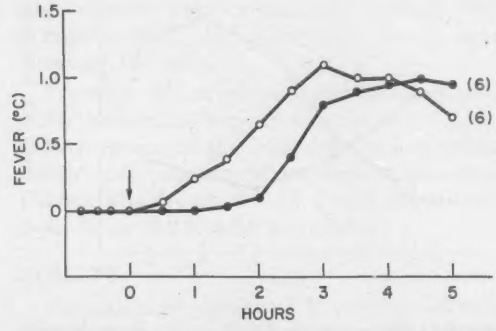

FIGURE 6.—Mean febrile responses of six endotoxin-tolerant rabbits (●———●) treated with 4 mg/kg of bleomycin and the mean fever of six normal rabbits treated with the same dose of bleomycin (○———○) (treatment given at 0 hours).

Endotoxin fever in animals given daily injections of bleomycin.—Although tolerance to the pyrogenic effect of bleomycin itself fails to develop with daily injections of the drug, rabbits receiving daily injections of bleomycin, nevertheless, may be tolerant to endotoxin. Rabbits that had been given five daily injections of 4 mg/kg of bleomycin were challenged on the sixth day with 0.3 μg of endotoxin. A group of normal rabbits were given the same dose of endotoxin on that day. There was no decrease in endotoxin-induced fever in the rabbits that had received daily bleomycin injections; in fact, rabbits pretreated with bleomycin had more fever than did normal controls ($P = 0.05$) (fig 7).

Bleomycin as a reticuloendothelial system (RES) blockading agent.—When given prior to endotoxin, the RES blockading agent thorium dioxide increases the sensitivity of normal rabbits to the pyrogenic properties of endotoxin. Moreover, when thorium dioxide or other RES blockading agents are given to endotoxin-tolerant rabbits, partial reversal of tolerance is noted the following day (15,20). Because of the increased febrile response to

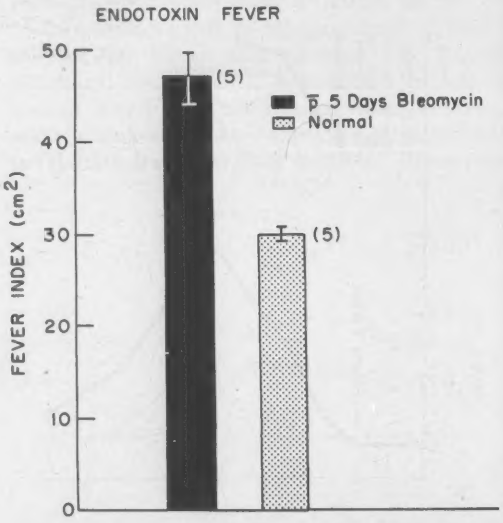

FIGURE 7.—Mean 5-hour fever indices (± standard error) in rabbits treated with 0.3 μg of *E. coli* endotoxin. Left bar: rabbits pretreated with five daily injections of bleomycin. Right bar: normal rabbits. There were five rabbits in each group.

endotoxin following bleomycin treatment, it was considered that bleomycin might have properties similar to those of RES blockading agents. Rabbits were made tolerant to endotoxin, and tolerance was confirmed on the sixth day with a test dose of 0.3 μg of endotoxin. The animals were then divided into two groups: six rabbits received 8 mg/kg of bleomycin iv 5 hours after the endotoxin injection while 4 rabbits received no drug and served as controls. The following day all rabbits were rechallenged with 0.3 μg of endotoxin. Bleomycin had no effect on the amount of endotoxin fever elicted.

Shwartzman reaction inducing properties of bleomycin.—Renal cortical necrosis as a manifestation of the generalized Shwartzman reaction follows the iv administration of two injections of endotoxin 12–24 hours apart. In addition, RES blockading agents have been used as the provocative (first) dose with the same results (21). In order to test bleomycin in the generalized Shwartzman reaction, 12 rabbits were given 15 mg of the drug iv, and 24 hours later six rabbits were given 40 μg of endotoxin iv. All rabbits were killed the next day. No evidence of renal cortical necrosis was seen in either group.

DISCUSSION

In these experiments, bleomycin has been shown to be a potent and consistent pyrogen. Using higher doses in rabbits, we have been able to demonstrate fever 1 hour after iv injection that resembles the acute fever seen in some patients with lymphoma who are treated with the drug (10–14). In some respects, the acute febrile reaction to this drug in patients resembles endotoxin fever. However, the data presented in this paper demonstrate that the pyrogenicity of bleomycin is not due to contaminating endotoxin. The characteristics of the febrile responses to the two agents are quite different. Endotoxin-induced fever begins 15–30 minutes after injection, rises quickly, and, depending on dose, is often biphasic. In contrast, bleomycin-induced fever begins only after a latent period of 1 hour and rises slowly. In addition, when doses of bleomycin that produce the same fever indices as endotoxin are used, the febrile responses are always monophasic.

Another striking difference between the reaction to bleomycin and the reaction to endotoxin is the change in wbc counts. After the administration of endotoxin there is a prompt leukopenia due to decreases in the number of polymorphonuclear leukocytes while the number of lymphocytes and monocytes remains unchanged (22). In the case of bleomycin-induced fever, the decrease in the wbc was due to decreases in both polymorphonuclear leukocytes and mononuclear leukocytes.

To further support our finding that the biologic properties of bleomycin are not due to contaminating endotoxin, we tested bleomycin in the limulus amebocyte lysate test. The results were consistently negative.

Finally, if endotoxin was responsible for the pyrogenicity of bleomycin, then rabbits tolerant to endotoxin should have been tolerant to bleomycin also. Such cross tolerance did not occur. Moreover, significant tolerance did not develop in rabbits receiving daily injections of bleomycin although the latent period was prolonged in animals after 6 days of treatment with the drug. It is of interest that daily injections of pyrogenic agents such as antigen in sensitized hosts or antigen-antibody complexes in normal subjects will produce tolerance (18,19,23). The failure of bleomycin to induce pyrogenic refractoriness thus differentiates it from most other pyrogens in the rabbit. In man, however, tolerance to bleomycin often develops after a few days (10–14,24,25).

Although tolerance to bleomycin did not develop in rabbits receiving daily injections of the drug, endotoxin fever in these rabbits was enhanced. In two experiments designed to investigate the possibility that this enhancement was due to the RES blockading properties of bleomycin, no such properties were apparent. Other evidence that bleomycin is distinct from endotoxin was provided by the drug's inability to serve as a preparative agent for the generalized Shwartzman reaction.

Despite the many differences noted in this paper between bacterial endotoxin and bleomycin, it appears that bleomycin fever is probably mediated by the same mechanism as other

experimental fevers, that is, through the release of endogenous pyrogen. In this study, we have demonstrated the presence of a rapid-acting pyrogen in the supernatant fluid of rabbit and human leukocytes incubated overnight with bleomycin as well as a similar transferable, rapid-acting pyrogen. Although direct action of bleomycin on the hypothalamus cannot be ruled out, it seems more likely that bleomycin releases endogenous pyrogen from host leukocytes in a manner similar to that of many other pyrogens (26).

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Effect of Pseudourea (NSC-56054) on DNA, RNA, and Protein Synthesis in Cultured Cells [1,2,3]

Jerry Z. Finklestein,[4,5] Paul Saarinen,[4] and Myron Karon [4,6]

SUMMARY

Cultures of hamster fibroblasts were exposed to pseudourea to investigate the effect of the drug on cell proliferation and synthesis of DNA, RNA, and protein. The drug inhibited the growth of the hamster cells and the synthesis of the three classes of macromolecules. There was no evidence of a selective effect.

[Cancer Chemother Rep 57:399–403, 1973]

Pseudourea is active against L1210 leukemia, prolonging the survival of BDF_1 mice bearing this tumor by 135%–190%.[7] The compound is currently in clinical trial, but it has been of limited use because of its photosensitizing properties (1,2). To investigate the mechanism of action of pseudourea in mammalian cells, we determined the effect of this compound on cell proliferation and synthesis of RNA, DNA, and protein in cultures of hamster fibroblasts.

MATERIALS AND METHODS

All studies were performed on stationary cultures of hamster fibroblasts (DON C cells). When maintained with modified McCoy's 5A medium, these cells grow with a doubling time of 13 hours (3).

Pseudourea was supplied as a 0.3% solution in propylene glycol. It was kept at −10 C and diluted with McCoy's medium immediately prior to use.

Effect of Pseudourea on Cellular Proliferation

Five-ml cultures in 2-oz prescription bottles were exposed to 0.1-, 1-, 10-, and 100-μg/ml doses of pseudourea. Six, 24, and 48 hours after the addition of the drug, cultures were detached with trypsin (0.75%) and diluted to 10 ml with saline. The cells were counted with a hemacytometer. Propylene glycol alone was added to replicate control cultures in amounts similar to those used for the drug solutions to determine whether the alcohol affected cellular proliferation. The effect of light on the rate of cell growth was studied by comparing cells treated with 10 μg/ml of pseudourea and kept in the dark with those grown 25 cm from a 40-watt daylight fluorescent bulb. The media were not changed during the course of these experiments, and cells were counted at 24 and 48 hours.

[1] Received May 12, 1971; revised June 25, 1973; accepted July 19, 1973.

[2] Supported in part by Public Health Service grants CA-11050, CA-02649, and CA-14560 from the National Cancer Institute (NCI), National Institutes of Health, Department of Health, Education, and Welfare, and by grant T-493 from the American Cancer Society.

[3] Pseudourea: CAS reg. No. 3063-89-6; pseudourea, 2,2'-(9,10-anthrylenedimethylene)bis[2-thio-, dihydrochloride, dihydrate. Supplied by Cancer Therapy Evaluation, Division of Cancer Treatment, NCI.

[4] Department of Pediatrics, University of Southern California, Los Angeles (Dr. Karon and Mr. Saarinen), and the University of California at Los Angeles (Dr. Finklestein).

[5] *Reprint requests to*: Jerry Z. Finklestein, MD, Department of Pediatrics, UCLA School of Medicine, Harbor General Hospital Campus, 1000 W Carson St, Torrance, Calif 90509.

[6] Scholar, Leukemia Society of America.

[7] Carter SK, Rall D, Schein P, et al. Clinical brochure: pseudourea, NSC-56054. Prepared by the National Cancer Institute, Bethesda, Md, 1968, 15 pp.

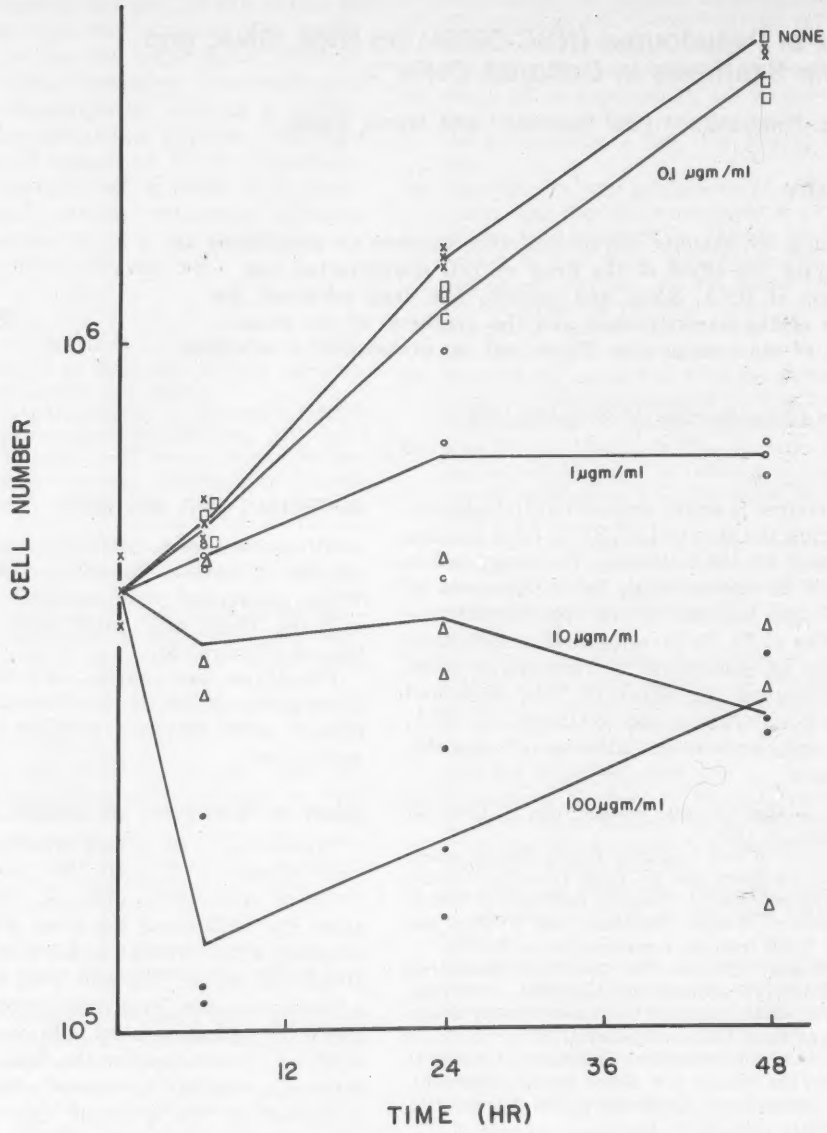

FIGURE 1.—Effect of pseudourea on cellular proliferation. Cultures of DON C fibroblasts were exposed to 0.1, 1, 10, and 100 µg/ml of pseudourea. Experiments were carried out in artificial light and triplicate cultures were used for each determination. Cell count is expressed as the total number of cells per 2-oz culture bottle.

Kinetic Experiments

Experiments on the kinetics of the synthesis of protein, DNA, and RNA were performed with cultures in 8-oz prescription bottles containing 15 ml of medium. Replicate cultures were treated with 100 μg/ml of pseudourea and incubated with 2 microcuries/ml of tritiated thymidine (specific activity, 6.7 curies/millimol), 2 microcuries/ml of tritiated uridine (specific activity, 1.3 curies/millimol), or 0.5 microcuries/ml of L-leucine ^{14}C (specific activity, 200 microcuries/millimol) for 1 hour at hourly intervals from zero time to 4 hours. After incubation, the cultures were washed twice with cold saline containing 100 μg/ml of nonradioactive thymidine, uridine, or leucine. The cells were detached with trypsin and washed once with saline containing the appropriate nonradioactive precursors. The cells were then diluted to a total volume of 5 ml with cold saline at 4 C, and a 0.5-ml sample was removed for cell count. The remaining 4.5 ml was diluted with 5% trichloroacetic acid (TCA) to a final volume of 45 ml and precipitated at 4 C for 30 minutes. The resulting precipitate was collected on glass-fibre paper (GF/C Whatman) and washed with cold 5% TCA. The filters were dried and assayed for radioactivity in a liquid scintillation counter using Liquiphor (Packard Instruments).

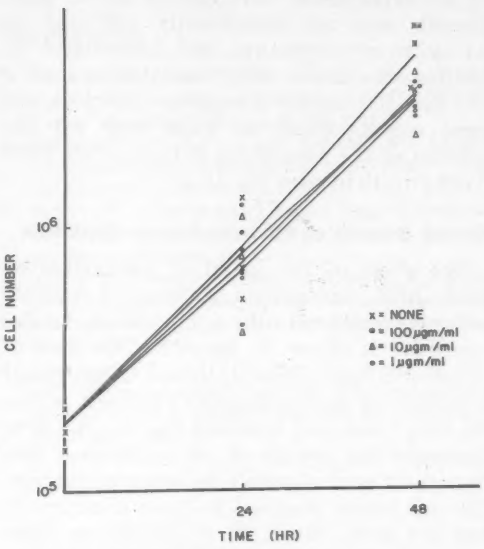

FIGURE 2.—Effect of propylene glycol on cellular proliferation. Cultures of DON C fibroblasts were exposed to various concentrations of propylene glycol, and their rate of cell proliferation was compared with that of an untreated control culture. Cell count is expressed as the total number of cells per 2-oz culture bottle. The concentrations of propylene glycol were adjusted to be those present when the concentration of pseudourea was either 1, 10, or 100 μg/ml.

RESULTS

Cell Proliferation

The effect of different concentrations of pseudourea on cell proliferation over a 48-hour period is shown in figure 1. Recovery of proliferative capacity was observed in the cultures exposed to 100 μg/ml of pseudourea. Since artificial light did not significantly alter the effect of pseudourea on DON C cell growth

TABLE 1.—Cell counts of cultures exposed to pseudourea (10 μg/ml) and grown either in light or in the dark

	No. of cells after—				
		24 Hrs		48 Hrs	
	0 Hrs	Light	Dark	Light	Dark
Control cultures	498,400	1,259,700	1,542,100	2,867,600	2,831,600
	431,400	1,339,800	1,446,900	3,415,600	2,922,700
	559,300	1,412,400	1,620,400	2,612,400	3,420,100
Treated cultures		574,300	308,800	375,400	462,900
		491,200	259,500	376,800	396,100
		408,800	193,100	281,600	420,300

after either 24 or 48 hours of exposure (table 1), all experiments were carried out in light. Growth was not significantly inhibited by 0.1 μg/ml of pseudourea, but dose-related inhibition was noted with concentrations of 1, 10, and 100 μg/ml. Propylene glycol in the same concentrations as those used for the solution of the pseudourea did not affect DON C cell growth in vitro (fig 2).

Kinetic Studies on Macromolecular Synthesis

The effect of 100 μg/ml of pseudourea on DNA, RNA, and protein synthesis in separate cultures treated with the appropriately labeled compound is shown in figure 3. This dose of pseudourea was chosen to insure a significant inhibition of macromolecular synthesis within the first 4 hours of exposure (fig 1). The drug depressed the uptake of all radioactive precursors by approximately the same percentage. The cell counts observed in these experiments had the same range of variability as those shown in figure 1 and table 1. The data are expressed as percent radioactive uptake relative to a control cell population. Because mean values were used to plot the results, individual points cannot be shown. Nevertheless, the slopes of the three curves are not significantly different. Thus, pseudourea does not preferentially affect the uptake of any of these radioactive precursors of DNA, RNA, or protein.

DISCUSSION

These data indicate that pseudourea inhibits the synthesis of DNA, RNA, and protein within 1 hour of exposure to a dose that carries an 80% cell lethality, ie, cell kill, at the end of 6 hours. Although DNA synthesis and RNA synthesis appear in figure 3 to be more profoundly inhibited than the protein synthesis, the difference is not significant. Therefore, the drug does not produce the "unbalanced growth" observed with more selective DNA inhibitors such as cytosine arabinoside (4).[8]

Pseudourea is known to have phototoxicity (ie, it injures cells in vivo in the presence of light) (1). However, in our study no significant enhancement of the cytotoxic effect was observed in vitro in cultures treated in the

[8] Cytosine arabinoside: NSC-63878; CAS reg. No. 69-74-9; cytosine, 1-β-D-arabinofuranosyl-, monohydrochloride.

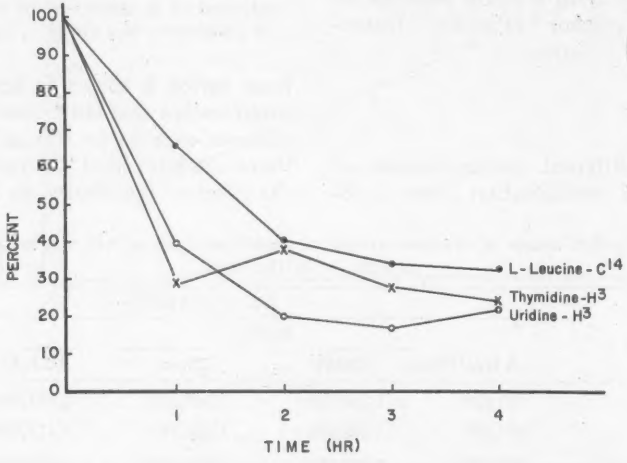

FIGURE 3.—Effect of 100 μg/ml of pseudourea on DNA, RNA, and protein synthesis. Each point represents the mean of three replicate cultures. The results are expressed as percent uptake of radioactive label in a control cell population.

presence of light and in cultures treated under artificial illumination as compared with cultures kept in the dark. Although the absorption maximum of pseudourea is in the ultraviolet range (260 nm), there is also significant absorption in the range of 350–405 nm. It is this latter absorption range that accounts for the phototoxicity of pseudourea (1). Since phototoxicity has been observed in patients kept indoors, failure to observe an effect on cell cytotoxicity in the presence of light may indicate that the phototoxicity and therapeutic effects of this drug are not necessarily related.

The mechanism of action of pseudourea has been studied in two bacterial systems by the Saunders (5). The synthesis of DNA and RNA was markedly inhibited by pseudourea, whereas the synthesis of protein was inhibited to a lesser degree. The drug interferes with DNA-dependent RNA polymerase and presumably forms intercalation complexes with DNA.

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Murine Neuroblastoma: A Model of Human Disease [1,2,3]

Jerry Z. Finklestein, Eitoku Arima, Patricia E. Byfield, John E. Byfield, and Eric W. Fonkalsrud [4,5]

SUMMARY

The C1300 murine neuroblastoma system was evaluated as a model of human disease. Drug toxicity studies were carried out in adult A/J mice. Cyclophosphamide, BCNU, and cytosine arabinoside prolonged the median lifespan of animals with palpable disease. Actinomycin D, methotrexate, 5-(3,3-dimethyl-1-triazeno)imidazole-4-carboxamide, and cyanocobalamin were inactive in the regimens used. The sensitivity of the murine neoplasm to cyclophosphamide correlates with clinical observations in children with active disease.

[Cancer Chemother Rep 57:405–412, 1973]

Neuroblastoma accounts for 8% of the deaths from cancer in children. Survival is influenced strongly by the patient's age, the extent of disease, and the degree of cell differentiation. An analysis of survival has been done in institutions affiliated with the Children's Cancer Study Group A (CCSGA). Although 92% of the infants with stage I disease survived free of tumor 2 years from the time of diagnosis, only 3% of the children more than 24 months old with stage IV disease survived for 2 years (1). More than 50% of the patients have stage IV disease at the time of original diagnosis; thus, for most of the patients, the outlook is extremely poor. To evaluate the impact of various modes of therapy on prognosis, a subcommittee of the National Cancer Institute compared the survival after treatment of patients treated in the prechemotherapy era with that of patients treated in the postchemotherapy era. This study showed that the survival pattern of children with and without metastases did not change between 1956 and 1962 (2). Additional evaluations performed by CCSGA investigators have revealed that survival has not improved significantly in the past decade.[6]

New approaches are needed to establish a logical basis for an organized program for the treatment of childhood neuroblastoma. In these new investigations, our present knowledge of the chemical control of other cancers should be applied in the perspective of the known clinical aspects of neuroblastoma. New drugs are usually evaluated in the murine L1210 leukemia

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[2] Supported in part by Public Health Service grants CA-14658 and CA-14560 from the National Cancer Institute (NCI) and HL-14333 from the National Heart and Lung Institute, National Institutes of Health, Department of Health, Education, and Welfare.

[3] BCNU and 5-(3,3-dimethyl-1-triazeno)imidazole-4-carboxamide were kindly supplied by Dr. Stephen K. Carter, Cancer Therapy Evaluation, Division of Cancer Treatment, NCI. Chemical nomenclature, NSC numbers, and CAS registry numbers for all compounds mentioned in this paper are listed in the List of Compounds at the end of the text.

[4] Departments of Pediatrics (Drs. Finklestein and P. Byfield) and Radiology (Dr. J. Byfield), University of California at Los Angeles-Harbor General Hospital, Torrance, Calif, and Department of Surgery (Drs. Arima and Fonkalsrud), UCLA Center for the Health Sciences, Los Angeles.

[5] The authors wish to thank Dr. John Weiner of the University of Southern California, Los Angeles, for performing the statistical evaluations; Mrs. Karen Tittle, Miss Connie Hsi, and Mrs. Carol Marks for providing excellent technical assistance; and Dr. Frank Hirose of the Department of Pathology, UCLA-Harbor General Hospital, for evaluating the biopsy material.

[6] Evans AE, and Liekin S. Personal communication.

system and one or more of the following animal tumor systems: murine P388 leukemia, murine melanoma B16; murine Lewis lung carcinoma, and Walker rat carcinosarcoma 256 (3). Methotrexate, actinomycin D, and mechlorethamine are active in these animal screening systems (3,4).

Despite the documented usefulness of these agents in other childhood cancers, the results of clinical trials of these drugs in children with neuroblastoma have been disappointing (5,6). These divergent results must surely be related to the specific characteristics of the neoplasms. The biologic properties of neuroblastoma in children are unique: (*a*) Neuroblastoma is a catecholamine-secreting neoplasm (7). (*b*) It may mature in vivo (8). (*c*) It has the highest rate of spontaneous regression associated with any human cancer (9). Thus, a more representative animal model should be evaluated. In this paper we report our initial studies with the murine C1300 neuroblastoma system and evaluate its potential as a model for the study of the disease in man.

The C1300 neuroblastoma cell line was isolated in 1940 by A. M. Cloudman as a spontaneous tumor from an A/J mouse. The tumor cells produce acetylcholinesterase and the enzymes necessary for the synthesis of neurotransmitters; under certain conditions, axon formation takes place (10). The variation of morphologic differentiation of the C1300 murine tumor in vitro (11,12) is similar to that of the human tumor in which histologic grading varies from a benign, well-differentiated ganglioneuroma form (grade I), which has an excellent prognosis, to the undifferentiated neuroblastoma (grade IV) (13).

MATERIALS AND METHODS

Neuroblastoma Lines

Culture and characteristics.—Murine neuroblastoma cells (originally obtained from Jackson Laboratories, Bar Harbor, Me) are cultured in Dulbecco's modified medium with 10% fetal calf serum. Two experimental substrains have been used. One line (wild type cells) is uncloned original stock. A second line (differentiating cells) (kindly supplied by Dr. H. Herschman, Center for the Health Sciences, UCLA School of Medicine, Los Angeles, Calif) has been serially cloned for accelerated axon development following reduction of serum concentration. The generation time in culture is 24 hours and is almost identical for the two lines. The therapy experiments reported in this paper were done with the differentiating line.

Tumor Induction

Adult A/J mice (more than 8 weeks of age) were obtained from Jackson Laboratories. Animals were quarantined for 2 weeks prior to admission into the study. The weights of the mice in any one experiment were within a 3-g range. The animals were inoculated subcutaneously (sc) in the interscapular area with 10^6 tumor cells cultivated in vitro.

Drug Toxicity and Chemotherapy Studies

These studies were performed with the following drugs: cyclophosphamide, vincristine, actinomycin D, methotrexate, BCNU, 5-(3,3-dimethyl-1-triazeno) imidazole-4-carboxamide (NSC–45388), cytosine arabinoside, and cyanocobalamin. All drugs were administered intraperitoneally (ip) to randomized groups of normal adult mice. Total leukocyte counts were performed three times a week. Therapy studies were carried out with the maximum nonlethal doses that caused myelosuppression. The drugs were administered ip either 30 minutes after the inoculation of tumor cells or when the sc implanted interscapular tumor measured 1.0–1.5 cm in diameter.

RESULTS

Tumor Induction

Sc inoculation of 10^6 neuroblastoma cells invariably causes fatal disease (table 1). Animals that had received 10^6 cells from the differentiating cell line had a tumor 1.0 cm in diameter in a median of 12 days (table 2). If it is assumed that a tumor that is 1.0 × 1.0 cm contains 10^9 cells, then the doubling time of tumor cells in vivo is approximately 1.3 days. This is similar to the doubling time in vitro. Biopsies of tumor revealed a predominantly undifferentiated malignant neoplasm consistent with neuroblastoma. It resembled Beckwith's grade IV stage of the human neoplasm (13).

TABLE 1.—Incidence of fatal disease in A/J mice inoculated sc with either wild type or differentiating C1300 cells

No. of cells inoculated	Incidence of fatal disease*	
	Wild type cells	Differentiating cells
10^5	13/13	4/5
10^4	8/8	20/20

*No. of deaths/No. of animals inoculated.

Toxicity and Chemotherapy Studies

Drug toxicity studies were conducted with adult A/J mice and various doses of the antitumor agents (table 3). Toxicity was evaluated on the basis of the myelosuppressive effect and lethality of the drug in normal adult mice. One dose of drug used for the therapy studies was either the maximum nonlethal dose or the LD10 dose. All groups of animals were treated with two or three regimens, one of which was reduced because we have found that animals with growing neoplasms are more sensitive to drugs than their non-tumor-bearing controls.

The effects of chemotherapy on palpable sc implanted neuroblastoma in A/J mice are outlined in table 4. Whereas cyclophosphamide, BCNU, and cytosine arabinoside reduced tumor volume, prolonged animal lifespan, and facilitated long-term survival in some mice, vincristine, actinomycin D, methotrexate, NSC-45388, and cyanocobalamin were not active with the doses and regimens used. The slight variability for median survival time depended on the day the drug was administered since in each experiment various treatment and control groups were matched for tumor size prior to the administration of the antitumor agent. Measurable tumors decreased by 0.1–0.6 cm with all doses of cytosine arabinoside even though this is not reflected in the survival data. The decreases in tumor size varied from 10% to 40%. Tumor reduction occurred within 48 hours after drug administration; regrowth was documented 2–3 days later. All doses were myelosuppressive in tumor-bearing animals, and total leukocyte counts decreased to less than 1000 leukocytes/mm^3.

Experiments in which the drugs were administered 30 minutes after the injection of 10^6 tumor cells are summarized in table 5. An increase of more than 50% in the median survival time was observed in only one of two experiments with cytosine arabinoside. Although cyclophosphamide and BCNU prolonged survival in animals with palpable disease, the drugs were inactive when administered at the same time as the tumor cells.

DISCUSSION

Therapy for childhood neuroblastoma may include surgical treatment, radiation therapy, and/or chemotherapy. Distant metastases are often present at the time of the original diagnosis, and definitive surgical treatment usually is not possible. Therefore, management of the tumor in children who have disseminated disease has been focused primarily on the chemotherapeutic approach with radiation therapy limited to specific localized areas of

TABLE 2.—Tumor development in A/J mice inoculated sc with wild type C1300 neuroblastoma cells*

No. of mice	No. of cells inoculated	Time (days) to the development of a 1.0 × 1.0-cm tumor	
		Range	Median
3	10^2	24–31	27
3	10^3	19–25	24
12	10^4	12–28	15
13	10^5	7–18	12
8	10^6	6–12	10

*The median time to the development of a 1.0 × 1.0-cm tumor in mice inoculated with 10^6 differentiating cells is 12 days.

TABLE 3.—Toxicity of chemotherapeutic agents in normal adult A/J mice

Drug	Dose* (mg/10 g, ip)	Incidence of toxicity (death)†	Day death occurred		Nadir wbc‡ (leukocytes/mm³)	Day nadir occurred
			Range	Median		
Cyclophosphamide	2.8 × 1	5/20	7–14	10	50	4
	2.5 × 1	6/10§	14–21	16	300	4
	2.3 × 1	0/10	—	—	4150	13
Vincristine	0.016 × 1	9/10	5–17	10	2900	13
	0.014 × 1	6/10§	2–21	6	500	4
	0.012 × 1	0/10	—	—	3700	13
Actinomycin D	0.0012, qd × 5	7/10	9–11	11	1700	14
	0.0008, qd × 5	0/10	—	—	3800	14
	0.0006, qd × 5	0/10	—	—	2550	8
Methotrexate	0.047, qd × 5	3/10	10–17	14	1100	11
	0.035, qd × 5	1/10	17	—	1200	15
BCNU	0.40 × 1	5/10	11–16	14	3400	14
	0.32 × 1	4/10	12–16	14	4250	14
	0.24 × 1	0/10	—	—	ND¶	—
	0.18 × 1	2/9	21–26	23	ND	—
NSC-45388	5.0 × 1	20/20	1–7	1	ND	—
	3.0 × 1	8/10	11–12	12	550	5
	2.25 × 1	0/10	—	—	500	12
	1.5 × 1	1/10	6	6	1500	7
Cytosine arabinoside	1.1, qd × 5	0/5	—	—	900	7
	0.9, qd × 5	0/5	—	—	1150	7
	0.7, qd × 5	1/10	7	7	400	7
	0.5, qd × 5	0/10	—	—	1000	7
	0.3, qd × 5	0/10	—	—	2700	7
	0.20, tid × 4	5/5	6–7	6	500	8
	0.15, tid × 4	0/5	—	—	1400	7
	0.10, tid × 4	1/5	10	10	3300	9
	0.05, tid × 4	0/5	—	—	4500	8

*qd = one injection daily; tid = three injections given 3 hours apart.
†No. of deaths/No. of animals treated.
‡The normal total leukocyte count for A/J mice is 5000–8000 leukocytes/mm³.
§Refers to two groups of mice that were inadvertently exposed to an insecticide spray.
¶ND = not determined.

disease (14,15). Many different therapeutic agents and schedules have been tried in children with neuroblastoma. The results have been very discouraging. Because they do not cause significant regression of measurable disease, the following agents are now rarely used: steroids, mechlorethamine, methotrexate, actinomycin D, daunomycin, uracil mustard, and melphalan (5,6,16–18). The lack of activity of vincristine in our animal screen may be due to the schedule used or it may indicate that the animal screen does not identify agents that have

TABLE 4.—Effect of chemotherapeutic agents on neuroblastoma in A/J mice with sc implanted tumors (1.0–1.5 cm)

Drug	Dose* (mg/10 g, ip)	No. of mice	Survival time† (days)		% ILS‡
			Range	Median	
Cyclophosphamide	Control	16	4–16	9	—
	2.5 × 1	17	21–38	27	200§
	2.0 × 1	17	7–27	16	77§
Vincristine	Control	13	6–13	9	—
	0.012 × 1	15	6–20	8	Neg
	0.01 × 1	15	4–17	8	Neg
Actinomycin D	Control	10	7–14	10	—
	0.0008, qd × 5	12	7–14	9	Neg
	0.0006, qd × 5	11	7–21	11	Neg
Methotrexate	Control	12	6–15	8	—
	0.035, qd × 5	14	8–13	8	Neg
	0.026, qd × 5	12	8–15	10	Neg
BCNU	Control	13	7–24	10	—
	0.24 × 1	13	13–29	20	100§
	0.18 × 1	13	13–84	20	100§
Cytosine arabinoside	Control	12	7–22	10	—
(Expt. 1)	0.17, tid × 4	7	7–21	7	Neg
	0.12, tid × 4	10	7–21	9	Neg
(Expt. 2)	Control	6	8–21	13	—
	0.17, qd × 4	6	8–21	12	Neg
	0.15, qd × 4	7	8–25	21	55§
	0.12, qd × 4	7	18–28	23	70§
(Expt. 3)	Control	11	8–19	11	—
	0.15, tid × 3	11	8–25	15	Neg
	0.12, tid × 3	11	4–42	15	Neg
NSC-45388	Control	13	5–25	11	—
	2.25 × 1	14	6–22	10	Neg
	1.5 × 1	14	3–29	13	Neg
Cyanocobalamin	Control	13	6–26	6.5	—
	0.012 × 1	15	3–17	6	Neg
	0.006 × 1	15	6–10	6	Neg

*Control mice received 0.2 ml of saline ip. qd = one injection daily; tid = three injections given 3 hours apart.

†Various treatment groups were matched according to tumor size prior to administration of the antitumor agent.

‡% ILS = percent increase in lifespan of treated animals over that of control animals (median survival time). Neg = ILS ≤ 50%.

§Values for cyclophosphamide were significant at $P < 0.00001$ for the 2.5-mg/10 g dose and $P = 0.006$ for the 2.0-mg/10 g dose. Values for BCNU and cytosine arabinoside were significant at $P = 0.016$. Values were analyzed by the method for exact probabilities for contingency tables described in: Maxwell, A. E. Analysing Qualitative Data. London, Methuen & Co., Ltd., 1961, pp 23–46.

TABLE 5.—Effect of chemotherapeutic agents on neuroblastoma in A/J mice when tumor cells and drug are injected on the same day

Drug	Dose* (mg/10 g, ip)	No. of mice	Survival time (days)		% ILS†
			Range	Median	
Cyclophosphamide	Control	5	14–26	24	—
	2.8 × 1	5	13–28	15	Neg
	2.5 × 1	5	13–28	26	Neg
	2.3 × 1	4	23–28	28	Neg
Vincristine	Control	5	14–26	24	—
	0.014 × 1	4	21–26	21	Neg
	0.012 × 1	5	3–26	19	Neg
	0.010 × 1	5	16–26	21	Neg
Actinomycin D (Expt. 1)	Control	5	16–21	18	—
	0.0012, qd × 5	5	7–9	7	Neg
	0.0008, qd × 5	5	9–28	25	Neg
	0.0006, qd × 5	5	9–25	9	Neg
(Expt. 2)	Control	5	23–25	23	—
	0.0012 × 1	5	7–18	25	Neg
	0.0008 × 1	5	23–28	25	Neg
	0.0006 × 1	5	23–35	28	Neg
Methotrexate	Control	5	16–21	18	—
	0.047, qd × 5	5	7–9	7	Neg
	0.035, qd × 5	5	7–25	23	Neg
	0.026, qd × 5	5	7–35	25	Neg
BCNU	Control	5	20–41	26	—
	0.3 × 1	5	20–33	25	Neg
	0.24 × 1	5	25–32	26	Neg
	0.18 × 1	5	22–32	25	Neg
Cyanocobalamin	Control	5	20–32	25	—
	0.012 × 1	5	18–32	29	Neg
	0.006 × 1	5	11–32	25	Neg
Cytosine arabinoside (Expt. 1)	Control	5	21–35	24	—
	0.17, tid × 3	5	27–44	35	Neg
	0.15, tid × 3	5	31–45	38	58‡
	0.12, tid × 3	4	27–71	40	66‡
(Expt. 2)	Control	10	18–35	21	—
	0.17, tid × 3	10	4–8	8	Neg
	0.15, tid × 3	10	21–30	28	Neg
	0.12, tid × 3	9	18–32	28	Neg

*Control mice received 0.2 ml of saline ip. qd = one injection daily; tid = three injections given 3 hours apart.
†% ILS = percent increase in lifespan of treated animals over that of control animals (median survival time). Neg = ILS ≤ 50%.
‡Not statistically significant.

borderline activity in man. Although vincristine has been used alone with limited success (19), most clinicians prefer to use cyclophosphamide with or without vincristine. Cyclophosphamide is now believed to be the only agent to which a significant number of children with metastatic disease will respond (5,20). Although neuroblastoma in children is sensitive to initial therapy with cyclophosphamide or radiation or both, there has not been a significant improvement in prognosis over the past two decades. During the same period of time, the prognosis for acute lymphocytic leukemia has improved strikingly. Although clinicians treating acute lymphocytic leukemia in children have had accurate animal models to work with, clinicians treating childhood neuroblastoma have not had accurate animal models.

In this study, the maximum safe dose for A/J mice of known cancer therapeutic agents was evaluated, and the response of the murine neoplasm to this dose was assessed. The initial pharmacologic data revealed that the alkylating agents cyclophosphamide and BCNU and the cell-cycle-specific agent cytosine arabinoside significantly prolonged the survival time of animals with palpable disease. The lack of activity in animals given the drug at the time of tumor cell implantation may be due to the particular schedule used. Cyanocobalamin was used because Bodian suggested in 1963 that children with neuroblastoma respond to therapy with this vitamin (21). Surveys by both American and English investigators have failed to confirm these observations (22,23). Our results for the alkylating agents are in keeping with clinical experience with the disease. Cytosine arabinoside has never been adequately evaluated in children with neuroblastoma; our studies suggest that these evaluations should be considered. The prolonged survival observed in the mice treated with the low dose of cytosine arabinoside may be caused by an immunologic mechanism. Heppner and Calabresi have reported (24) that small doses of cytosine arabinoside will decrease the ability of the serum to block lymphocyte-mediated tumor immunity in C3H/HeJ mice that have mammary tumors. They found that a small dose of drug depressed tumor growth, whereas treatment with a larger dose also depressed cell-mediated immunity and had no antitumor effect. Tumor immunity appears to exist in children who have neuroblastoma (25). If this is true for the murine tumor, and if the prolongation of lifespan observed with the low dose of cytosine arabinoside was caused by an immunologic mechanism, then the murine model should be an excellent system for the evaluation of combination chemotherapy and immunotherapy.

A valid model for the screening of therapeutic regimens should demonstrate that each therapeutic regimen is effective in both the mouse and man. The greater the difference between the two systems, the less valid the model becomes in predicting human tumor response. The fact that prolongation of survival (which does not occur in man) is used as an index of activity in the murine system should not detract from the potential usefulness of the system as a screen for drug activity in human disease since the preliminary evaluations reported here with the animal model have identified the activity of the alkylating agents. In addition, the experiments performed thus far indicate that agents known to be active in other animal tumors (eg, actinomycin D and methotrexate) but inactive in childhood neuroblastoma are inactive in the murine neuroblastoma system in the doses and regimens used. Detailed studies of different regimens are necessary to complete the evaluation of the agents used in these studies. Studies including experiments with treatment schedules (for all agents) similar to those described by Venditti (26) for L1210 leukemia are underway.

Since more than 50% of patients with neuroblastoma system in the doses and regimens of original diagnosis, chemotherapy provides the main hope for improvement in the prognosis of this disease. However, chemotherapy is inadequate at present. Our evaluation indicates that the murine C1300 neuroblastoma screen may provide a reliable model which can reasonably be expected to lead to an improvement in the treatment and prognosis of childhood neuroblastoma.

LIST OF COMPOUNDS

Actinomycin D: NSC-3053

BCNU: NSC-409962; CAS reg. No. 154-93-8; urea, 1,3-bis(2-chloroethyl)-1-nitroso-

Cyanocobalamin: NSC-80365; CAS reg. No. 68-19-9; vitamin B_{12}

Cyclophosphamide: NSC-26271; CAS reg. No. 6055-19-2; 2*H*-1,3,2-oxazaphosphorine, 2-[bis(2-chloroethyl)amino]tetrahydro-, 2-oxide, monohydrate

Cytosine arabinoside: NSC-63878; CAS reg. No. 69-74-9; cytosine, 1-β-D-arabinofuranosyl-, monohydrochloride

Daunomycin: NSC-82151; CAS reg. No. 23541-50-6; daunorubicin (NSC-83142)

Mechlorethamine: NSC-762; CAS reg. No. 55-86-7; diethylamine, 2,2'-dichloro-*N*-methyl-, hydrochloride; HN2; nitrogen mustard

Melphalan: NSC-8806; CAS reg. No. 3223-07-2; alanine, 3-[*p*-[bis(2-chloroethyl)amino]phenyl]-, monohydrochloride, L-; L-sarcolysin

Methotrexate: NSC-740; CAS reg. No. 59-05-2; glutamic acid, *N*-[*p*-[[(2,4-diamino-6-pteridinyl)methyl]methylamino]benzoyl]-

NSC-45388: CAS reg. No. 4342-03-4

Uracil mustard: NSC-34462; CAS reg. No. 66-75-1; uracil, 5-[bis(2-chloroethyl)amino]-

Vincristine: NSC-67574; CAS reg. No. 2068-78-2; leurocristine, sulfate (1:1)

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Preliminary Trials With a Combination of Adriamycin (NSC-123127) and Bleomycin (NSC-125066) in Adult Malignancies [1,2,3]

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SUMMARY

Sixteen adult patients with metastatic malignant disease were treated with 4-day courses of adriamycin at a dose level of 15 mg/m²/day given intravenously (iv) every 3 weeks in combination with twice weekly bleomycin at a dose level of 15 mg/m²/day given iv. Ten of the 16 patients responded. The responses were observed in five of 11 patients with squamous cell tumors, two of two with hepatomas, one of one with adrenal cell adenocarcinoma, one of one with transitional cell carcinoma of the bladder, and one of one with bronchiolar carcinoma of the lung. Improvement was recorded in three additional patients with squamous cell carcinoma. The principal side effects were leukopenia and mucositis from which the patients recovered rapidly despite the continuation of bleomycin. The combination was otherwise well tolerated and appears to be worthy of more extensive trials. Considerations for a biweekly dose schedule of adriamycin are discussed.

[Cancer Chemother Rep 57:413–418, 1973]

Adriamycin is an antibiotic isolated in 1967 from a mutant species of *Streptomyces peucetius* (1). It has been demonstrated to have activity against a number of human neoplasms, including squamous cell carcinomas (1,2), sarcomas (1,3), breast (1,2) and lung (4) carcinomas, lymphomas (1,3), and acute leukemias (1,3). Preliminary studies with adriamycin in man have utilized 3–4-day courses given every 3–4 weeks (1–3). The acute dose-limiting toxic effect is usually leukopenia, although thrombocytopenia or oral mucositis and ulceration can be the dominant side effects (1–3). Multiple administrations of the drug to cumulative doses in excess of 550 mg/m² is attended by a sharply rising incidence of severe cardiac toxicity (2–5).

Bleomycin was isolated from *Streptomyces verticillus* in 1966 (6). It has been demonstrated to have activity against a variety of tumors, including testicular carcinomas (7,8), squamous cell carcinomas (7–11), lymphomas

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[3] Adriamycin and bleomycin were supplied by Cancer Therapy Evaluation, Division of Cancer Treatment, NCI. Chemical nomenclature, NSC numbers, and CAS registry numbers for all compounds mentioned in this paper are listed in the List of Compounds at the end of the text.

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(7,8,12), and Hodgkin's disease (7,12). The optimal dose schedules and routes of administration of bleomycin have not yet been delineated; however, a twice-weekly schedule has been found to be well tolerated (8). There is little, if any, bone marrow toxicity (7,8,13). The dose-limiting toxic effect appears to be pulmonary interstitial fibrosis which tends to become prominent at cumulative doses in excess of 200 mg/m² (7,8,14). The present investigation was undertaken to determine if these two agents, with their differing toxic effects and similar activities in a number of human tumors, could be safely combined in commonly used dose schedules and still maintain significant antitumor activity. Additional limited experience with both agents used singly is also reported.

PATIENTS AND METHODS

Sixteen adult patients with biopsy-proven metastatic malignant disease were selected for treatment with a combination of adriamycin and bleomycin. One additional patient received adriamycin alone, and six patients were treated with bleomycin alone. All patients had measurable progressive disease. Adriamycin was administered intravenously (iv) at a dose level of 15 mg/m² for 4 consecutive days; courses were repeated at 3-week intervals. Bleomycin was administered iv at 15 mg/m² on the 1st and 4th days of each week beginning on the 1st day of treatment with adriamycin. Treatment was terminated if no change was apparent in the measurable disease after 6 weeks of therapy. Therapy was also discontinued in patients who had progressive disease at any time after the first 3 weeks of treatment. Those patients who showed improvement after 6 weeks of treatment continued to receive therapy until their improvement had stabilized, at which time they entered into the maintenance phase. The maintenance phase consisted of the first 10 days of the above regimen followed by 0.1 mg/kg/day of chlorambucil given orally for 6 weeks, a 1-week rest, and recycling to the first 10 days of the adriamycin and bleomycin regimen.

If the white blood cell count (wbc) fell below 1000 cells/mm³ or the platelet count fell below 25,000 platelets/mm³ after any course of adriamycin, the total adriamycin dose in the subsequent courses was reduced by 25%. If the wbc and platelet counts were initially below 10,000 cells/mm³ and 500,000 platelets/mm³, respectively, and did not fall below 5000 cells/mm³ or 200,000 platelets/mm³, then the dose of adriamycin was increased to 20 mg/m² in subsequent courses.

The product of the largest cross diameters of the lesions was used as the sole criterion to evaluate the response to therapy. A complete remission (CR) was defined as complete disappearance of all measurable evidence of disease. A partial remission (PR) was defined as at least a 50% decrease in the size of all measurable lesions. Improvement (I) was considered to be a 25%–50% decrease in the size of the lesions. A change of less than 25% was considered to be no change (NC). Progressive disease (PD) was defined as the appearance of a new lesion or an increase of more than 25% in the size of existing lesions.

All patients were followed twice a week with complete blood counts and once a week with hepatic and renal function tests. An electrocardiogram was obtained prior to each course of adriamycin; if any change was noted the drug was withheld. Lesions were measured at least once a week.

Side effects were scored on a scale from zero to 4, with zero being normal. The designations 1 to 4 were defined for each parameter as follows:

Skin:
1 = erythema or edema or both
2 = blisters
3 = ulcerations
4 = sloughing

Infection:
1 = fever for more than 24 hours with no organism isolated
2 = local infection with organism recovered
3 = sepsis
4 = fatal

White blood cell count (cells/mm³):
1 = < 4500
2 = < 3500
3 = < 2500
4 = < 1500

Platelet count (platelets/mm³):

1 = < 125,000
2 = < 100,000
3 = < 50,000
4 = < 20,000

Pulmonary:

1 = rales
2 = rales and restrictive disease and/or decreased diffusion capacity
3 = mild infiltrates on roentgenogram
4 = coalescence of infiltrates

RESULTS

The results of therapy with the combination of adriamycin and bleomycin are summarized in table 1. Objective responses (CR and PR) were observed in ten of 16 patients; three additional patients were considered improved. There were 11 patients with squamous cell carcinoma (cervix, head and neck, and esophagus). Five of these patients responded to therapy and three additional patients improved. There was no response to therapy in three of these patients. Responses were also observed in two of two patients with hepatomas, one of one with adrenal adenocarcinoma, one of one with bronchiolar carcinoma, and one of one with transitional cell carcinoma of the bladder. One additional patient with a transitional cell

TABLE 1.—Summary of clinical responses*

Diagnosis, site	Previous chemotherapy	Response	Time to start of response (days)	Duration of response (days)
Squamous cell carcinoma				
larynx	HU	NC	43	RFS
larynx	none	PR	17	36+†
retromolar trigone	none	PR	18	28
floor of mouth	none	PD	45	RFS
pyriform sinus	none	I	53	35
lateral pharnyx	none	PR	24	35
epiglottis	none	I	26	10+†
esophagus	none	I	22	30
cervix	none	CR	18	68
cervix	none	PD	56	RFS
disseminated‡	VCR, PRED	PR	5	65
Bronchiolar, lung	HMM, AC, B, 6-MP	PR	36	27
Adenocarcinoma, adrenal	AC, B, C, M, 5-FU, HMM	PR	11	82
Hepatoma	C, 6-MP, HMM, AC, B	PR	14	305
Hepatoma	none	PR	37	4+§
Transitional cell, bladder	none	PR	9	12

*Abbreviations: HU = hydroxyurea; VCR = vincristine; PRED = prednisone; HMM = hexamethylmelamine; AC = cytosine arabinoside; B = bleomycin; 6-MP = 6-mercaptopurine; C = cyclophosphamide; M = methotrexate; and 5-FU = 5-fluorouracil. NC = no change; PR = partial remission; PD = progressive disease; I = improvement; and CR = complete remission. RFS = removed from study.

†Lost to followup.

‡Lymphoepithelioma.

§Patient died between Days 271 and 302 after the start of therapy.

carcinoma of the bladder was treated with adriamycin alone and had a partial response. Six additional patients were treated with bleomycin alone. Three of these patients with Hodgkin's disease improved; disease in the remaining three patients progressed (one patient with poorly differentiated nodular lymphoma, one with renal cell adenocarcinoma, and one with angiosarcoma). The mean duration of therapy required to achieve a complete or partial response with the bleomycin and adriamycin combination was 22 days (range 5–37), and the mean duration of the response was 66 days (range 12–305 days).

The dose level of adriamycin was reduced in three of the patients because leukopenia developed. The dose level was increased to 20 mg/m²/day in only two patients. Bleomycin was discontinued in five of the patients because of the onset of signs of pulmonary toxic effects after they had received total doses of 75, 165, 180, 210, and 350 mg/m², respectively. The maximum drug toxicity observed in the 42 courses of adriamycin and bleomycin given to the 16 patients is tabulated in table 2. The period of severe leukopenia in four of the patients and in six courses of the drugs was short, lasting from 1 to 5 days. Mucositis, sometimes with oral ulcerations, was the most severe side effect; it occurred in all of the patients and during 26 of the 39 evaluable courses. It was not necessary to discontinue bleomycin in any patient during the period of adriamycin-induced bone marrow suppression or mucositis. Alopecia occurred in nearly every patient. Two patients had reversible elevations of blood urea nitrogen levels (from 10 to 26 mg/100 ml, and from 22 to 42 mg/100 ml, respectively). One patient experienced paresthesias in the fingertips. One patient with a hepatoma had an unexplained grand mal seizure on Day 39 of therapy; he has now been in the study for over 215 days without further seizures. No cardiac toxicity was observed, however no patient had received more than 550 mg/m² of adriamycin.

By observing the patients from one to two times per week it was frequently possible to gain an impression of individual drug effects upon the observed improvements and responses. Six of the responding patients relapsed during the end of the period of recovery from adriamycin side effects, ie, Days 15–21 of the treatment cycle. Two patients relapsed when bleomycin had to be discontinued due to pulmonary toxic effects. The remaining two responding patients relapsed while receiving chlorambucil. The courses of the six nonresponding patients were too smooth to distinguish a possible single-drug effect.

DISCUSSION

In the present investigation, the combination of bleomycin with adriamycin was found to be well tolerated. The primary significant side effects included severe leukopenia during six courses of therapy in four of the patients, and mucositis in all of the evaluable patients. These side effects were felt to be due to the adriamycin treatment since the patients recovered quickly despite the continuing bleomycin treatment during the period of maximum leukopenia and mucositis. The response rate (CR and PR) of ten of 16 patients was encouraging, especially in squamous cell car-

TABLE 2.—Summary of toxic effects

Toxic effect	Degree*					
	0	1	2	3	4	Not evaluable
	No. of patients (No. of courses)†					
Skin	10 (27)	5 (9)	1 (2)	0 (0)	0 (0)	0 (4)
Infection	10 (35)	4 (2)	1 (2)	0 (0)	0 (0)	1 (3)
Wbc	2 (12)	1 (5)	3 (11)	6 (4)	4 (6)	0 (4)
Platelet count	12 (34)	2 (2)	2 (2)	0 (0)	0 (0)	0 (4)
Pulmonary	11	3	2	0	0	0

*See text, Patients and Methods, for definitions.
†Courses refer to each 21-day cycle of therapy.

cinoma where five of 11 patients responded and three additional patients showed improvement. Using a different dose schedule, Cortes et al (15) reported responses in four of eight patients with head and neck carcinoma. The combination has also been used for gynecologic tumors (16). Reported response rates to adriamycin alone in patients with squamous cell carcinoma have ranged from 20% to 50% (1,2), and for bleomycin alone from 12% to 93% (7–11), with patients who had well-differentiated lesions responding more favorably than those with undifferentiated lesions (9,10). Although it is not possible to directly compare those response rates with the ones reported here, the data suggest that the combination is worthy of more extensive study. The responses of the two patients with hepatomas are also of interest. To our knowledge, bleomycin has not previously been utilized in this disease, however, no response to adriamycin alone has been reported in three patients with hepatomas (2,3).

The short durations of the remissions observed in this study and in that of Cortes et al (15) are disturbing, and suggest that these drugs, either alone or in combination, while useful for obtaining remissions, may need to be used in a different schedule or with other drugs to maintain the remission. The study reported here was discontinued because of the short remission durations and because of the observation that the majority of patients were relapsing during the end of the recovery period from adriamycin side effects. This latter observation, as well as the recent information that the drug appears to be most efficient at killing cycling cells in the S phase (17,18), prompted us to explore a twice-weekly adriamycin schedule. Such a schedule has been used as the maintenance therapy for the patient with a hepatoma who relapsed after 319 days. Bleomycin was discontinued in this patient because of early pulmonary toxic effects after the patient had received a total dose of 250 mg/m^2. Two patients with squamous cell carcinomas have since been treated with twice-weekly adriamycin and bleomycin and have entered complete remissions. As a result of these experiences we are currently evaluating this latter dose schedule more intensively.

ADDENDUM

Since submitting this manuscript, we have obtained a partial remission in a third patient with a hepatoma using the combination reported here. At present he has been under treatment for 188 days.

LIST OF COMPOUNDS

Adriamycin: NSC-123127

Bleomycin: NSC-125066

Chlorambucil: NSC-3088; CAS reg. No. 305-03-3; butyric acid, 4-[*p*-[bis(2-chloroethyl)amino]phenyl]-

Cyclophosphamide: NSC-26271; CAS reg. No. 6055-19-2; 2*H*-1,3,2-oxazaphosphorine, 2-[bis(2-chloroethyl)-amino]tetrahydro-, 2-oxide, monohydrate

Cytosine arabinoside: NSC-63878; CAS reg. No. 69-74-9; cytosine, 1-β-D-arabinofuranosyl-, monohydrochloride

5-Fluorouracil: NSC-19893; CAS reg. No. 51-21-8; 5-FU

Hexamethylmelamine: NSC-13875; CAS reg. No. 645-05-6

Hydroxyurea: NSC-32065; CAS reg. No. 127-07-1

6-Mercaptopurine: NSC-755; CAS reg. No. 6112-76-1; purine-6-thiol, monohydrate; 6-MP

Prednisone: NSC-10023; CAS reg. No. 53-03-2; pregna-1,4-diene-3,11,20-trione, 17,21-dihydroxy-

Vincristine: NSC-67574; CAS reg. No. 2068-78-2; leurocristine, sulfate (1:1)

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Study of Methyl-CCNU (NSC-95441) in the Treatment of Lung Cancer [1,2,3]

Frederick Richards II, Thomas F. Pajak, M. Robert Cooper, and Charles L. Spurr [4,5]

SUMMARY

Twenty-five patients who had carcinoma of the lung were treated with orally administered methyl-CCNU at a dose level of 150 mg/m² of body surface area every 6 weeks. Objective responses were noted in four patients (16%). Methyl-CCNU favorably influenced survival in the responders. The toxicity was moderate and toxic reactions were mainly nausea and vomiting (within 1–2 hours of drug administration) and bone marrow depression (within 2–5 weeks).

[Cancer Chemother Rep 57:419–422, 1973]

Two nitrosourea derivatives, BCNU and CCNU, have been shown to be useful in bronchogenic carcinoma (1).[6] Another nitrosourea derivative, methyl-CCNU, has proved to be more effective than CCNU and BCNU against the advanced Lewis lung tumor in achieving a high percentage of cures (2). The mechanism of action of methyl-CCNU is unknown but it probably acts as an alkylating agent and inhibits several key enzymatic steps in the formation of DNA.[7]

The purpose of this pilot study was to evaluate the effectiveness of methyl-CCNU in the treatment of patients with either inoperable, recurrent, or metastatic bronchogenic carcinoma.

MATERIALS AND METHODS

This study comprised 25 patients who had histologically proven carcinoma of the lung with at least one measurable malignant lesion. The disease was confined to one lung in only two patients. The other patients had distant or contralateral metastases or both. There was a treatment-free observation period of at least 3 weeks for patients who had had previous surgical treatment, radiotherapy, or chemotherapy.

The therapeutic regimen consisted of methyl-CCNU administered orally and repeated at 6-week intervals. Methyl-CCNU was supplied in 10-, 20-, 50-, and 100-mg capsules. The dose level was 150 mg/m² of body surface area given

[1] Received Mar 27, 1973; revised July 2, 1973; accepted July 19, 1973.

[2] Supported in part by Public Health Service grants T12-8037 and CA-12197 from the National Cancer Institute (NCI), National Institutes of Health, Department of Health, Education, and Welfare; and the Forsyth Cancer Service.

[3] Methyl-CCNU: CAS reg. No. 33073-59-5; urea, 1-(2-chloroethyl)-3-(4-methylcyclohexyl)-1-nitroso-. Supplied by Drug Research and Development, Division of Cancer Treatment, NCI.

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[6] BCNU: NSC-409962; CAS reg. No. 154-93-8; urea, 1,3-bis(2-chloroethyl)-1-nitroso-.

CCNU: NSC-79037; CAS reg. No. 13010-47-4; urea, 1-(2-chloroethyl)-3-cyclohexyl-1-nitroso-.

[7] Broder LE, and Carter SK. Clinical brochure: 1-(2-chloroethyl)-3-(4-methylcyclohexyl)-1-nitrosourea (Me-CCNU) NSC-95441. Prepared by the NCI, Bethesda, Md, Feb 1972, 51 pp.

over a period of 10–30 minutes depending on the size of the capsules. Both a pretreatment white blood cell count (wbc) of 4500 cells/mm³ or more and a platelet count of 120,000 platelets/mm³ or more were requisite for each patient to enter the study. Subsequent courses of therapy were modified by the nadir of the wbc or the platelet count or both during each preceding 6-week treatment interval according to the following schedules. If the wbc dropped below 3000 cells/mm³ or the platelet count dropped below 120,000 cells/mm³ during the last preceding treatment interval, the dose level was reduced by 25%. If the wbc dropped below 2000 cells/mm³ or the platelet count dropped below 50,000 cells/mm³, the dose level was reduced by 50%. If the wbc remained less than 4500 cells/mm³ or the platelet count remained less than 120,000 cells/mm³, the drug was withheld entirely, pending the recovery to these higher levels.

Pretherapy baseline evaluation of the patient included chest roentgenogram; complete blood cell count, platelet count, and reticulocyte count; total serum bilirubin, calcium, phosphorus, albumin, total protein, blood urea nitrogen, glucose, uric acid, alkaline phosphatase, and glutamic oxaloacetic transaminase (SGOT) determinations; urinalysis; and histologic diagnosis. Patients were seen at 1–2-week intervals for evaluation of drug toxicity and response to chemotherapy.

The results of chemotherapy were evaluated on the basis of objective tumor regression (longest times widest perpendicular diameter), using the following criteria. Complete response—complete disappearance of all measurable disease; partial response (good)—regression of measurable tumor by 50% or more; partial response (fair)—regression of measurable tumors by 25%–49%; static response—no progression or less than a 25% regression; mixed response—regression of measurable tumor by 50% or more with progression, or no change in other measurable tumors; no response—progression of disease.

RESULTS

Twenty-five patients received one to five courses of the methyl-CCNU treatment. Most of the patients had either adenocarcinoma or oat cell carcinoma (table 1). Eighteen of the 25 patients were men and seven were women. The median age was 56 years.

Prior to treatment with methyl-CCNU, six patients had had radical surgical treatment with intent to cure. Twelve patients had received prior radiation therapy to their primary lesion, and three patients had received prior chemotherapy.

At the beginning of chemotherapy, disease limited to one lung was present in two patients; both had pleural effusion containing malignant cells. The remaining 23 patients had advanced carcinoma with systemic symptoms. Eleven patients had metastases to the opposite lung, ten had supraclavicular or axillary nodes, three had subcutaneous nodules, nine had liver metastases, five had brain metastases, and 11 had bone metastases.

TABLE 1.—Histologic diagnosis and response in 25 patients who received methyl-CCNU therapy

Histologic type	No. of patients*	No. of men/ women	No. of responders	Response and duration
Adenocarcinoma	9 (2)	6/3	2	Fair, 6 wks/fair, 4 wks
Oat cell carcinoma	7 (2)	6/1	2	Good, 8 wks/good, 10 wks
Squamous cell carcinoma	5 (2)	5/0	0	—
Anaplastic carcinoma	3	1/2	0	—
Alveolar cell carcinoma	1	0/1	0	—
	25	18/7	4	

* Figures in parentheses indicate the number of patients who received only one course of chemotherapy.

Six of the 25 patients received only one course of methyl-CCNU therapy. All six of these patients died of progressive disease within 5 weeks after the start of chemotherapy. Seven patients received only two courses because of rapid progression of disease or death. The remaining twelve patients received three or more courses of methyl-CCNU.

Tumor regression of 25% or more was obtained in four patients (table 1). In another patient with oat cell carcinoma, a mixed response was obtained (75% regression of hilar lesion, but 25% progression of peripheral lesion). One patient with squamous cell carcinoma and another patient with anaplastic carcinoma, with rapidly advancing disease, had static responses for a period of 16 and 11 weeks, respectively.

The average time lapse from diagnosis to treatment with methyl-CCNU was 17 weeks. At the time of this paper, 22 of the 25 patients are dead and three are still alive with their disease. The two patients who had a static response and the patient who had a mixed response are grouped together with the four other responders. The survival curve comparing the seven responders with the 18 nonresponders is presented in figure 1. The median duration of survival for responders was 19.3 weeks and for the nonresponders was 5.6 weeks from the start of treatment. The manifestations of response in all patients became evident within 2 weeks from the initiation of chemotherapy. The median of the remission duration was 10.4 weeks.

Nausea and vomiting occurred in four patients within 1–5 hours. All patients received either 10 mg of prochlorperazine or 25 mg of chlorpromazine given parenterally before each dose. Depression of peripheral leukocyte and thrombocyte counts occurred in seven patients; in three patients the wbc fell to less than 2000 cells/mm^3 or the platelet count fell to less than 50,000 platelets/mm^3 or both.

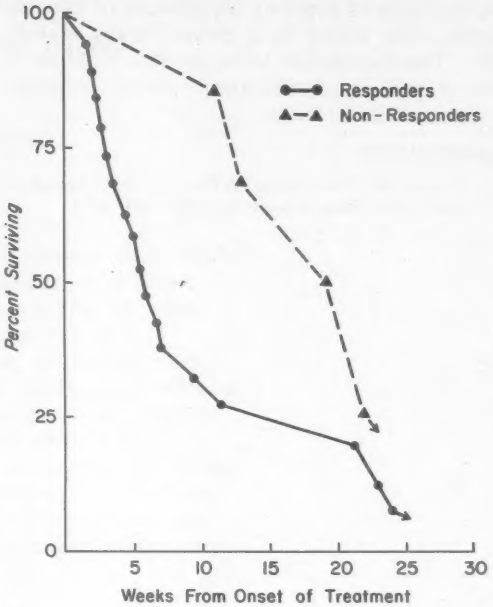

FIGURE 1.—Survival curve comparing the seven responders with the 18 nonresponders.

DISCUSSION

It is difficult to compare our results with those of others in the treatment of bronchogenic carcinoma, but such a comparison between various drugs from different series has been made (3). Comparison between different clinical trials is difficult because of differences in the study population, the design of the clinical trial, and the histology of the tumor.

Our pilot study established that methyl-CCNU is effective in the treatment of bronchogenic carcinoma because it resulted in a significant survival gain for the responders. The two patients who had disease limited to one lung were both nonresponders but had the longest survival. The other long-term survivor who was a nonresponder has subsequently responded to adriamycin and also to combination therapy with vincristine and methotrexate.[8]

[8] Adriamycin: NSC-123127.
Vincristine: NSC-67574; CAS reg. No. 6449-03-2; leurocristine, sulfate (1:1).
Methotrexate: NSC-740; CAS reg. No. 59-05-2; glutamic acid, *N*-[*p*-[[(2,4-diamino-6-pteridinyl)methyl]-methylamino]benzoyl]-.

Methyl-CCNU was selected for clinical trial on the basis of superior suppression of the rat Lewis lung tumor in a chemotherapy screen (2). The correlative value of this predictive screen will be of continued interest in more extensive clinical trials.

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Phase II Study of Emetine (NSC-33669) in the Treatment of Solid Tumors [1,2]

Saleem Siddiqui, Dincer Firat, and Seymour Olshin [3,4]

SUMMARY

Fifty-one patients with various advanced solid tumors were treated with subcutaneous injections of emetine at a dose level of either 1 mg/kg of body weight daily for 10 days or 1.5 mg/kg of body weight twice weekly for 5 weeks repeated at intervals of 3 weeks. The toxicity was moderate and reversible, consisting mainly of muscular weakness, local pain, and transient cardiac arrhythmias. No complete or partial regression of tumor was observed. Emetine at the dose level and schedule of administration used in this study appeared to be inactive in bronchogenic carcinoma and demonstrated no response in eight patients with gastrointestinal carcinoma, six with breast carcinoma, nine with genitourinary carcinoma, six with gynecologic carcinoma, and six with miscellaneous malignancies.

[Cancer Chemother Rep 57:423–428, 1973]

Emetine is the active alkaloid of ipecac, extracted from the root of the plant *Cephaëlis ipecacuanha*, which has been in therapeutic use for over 3 centuries (1). It has been administered parenterally only since 1912, principally in the treatment of amebiasis.[5] The drug has also been used for treatment of various bacterial as well as viral infections (2,3).

The use of emetine in the treatment of human malignancies also dates back to the beginning of the century. The dose levels were frequently excessive and the results inconclusive (4,5). The interest in emetine as an antineoplastic drug was renewed after the scattered reports of its efficiency in the treatment of nonspecific granulomas and certain animal and human cancers (6–10).

Emetine is a strong inhibitor of protein synthesis, its mechanism of action being the prevention of the translation of amino-acyl transfer RNA to ribosomal peptide. It also inhibits in vitro aerobic glycolysis in cardiac muscle.[5]

The purpose of this study is to evaluate the effectiveness of emetine in a larger group of patients with various solid tumors using two different dose schedules.

MATERIALS AND METHODS

Fifty-one patients with various histologically

[1] Received Apr 4, 1973; revised June 21, 1973; accepted July 19, 1973.

[2] Emetine: CAS reg. No. 316–42–7; emetine, hydrochloride. Supplied by Cancer Therapy Evaluation, Division of Cancer Treatment, National Cancer Institute (NCI).

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[5] Livingston RB, and Carter SK. Clinical brochure: emetine hydrochloride. Prepared by the NCI, Bethesda, Md, Jan 1970, 28 pp.

proven solid tumors who had at least one measurable malignant lesion were included in the study. All patients had inoperable, recurrent, or metastatic cancer which was considered to be either unresponsive or no longer responsive to conventional chemotherapy or radiotherapy. There was a minimum of a 4-week treatment-free period between the emetine treatment and previous chemotherapy or radiotherapy. None of the patients had received prior therapy with emetine.

The pretherapy baseline evaluation included chest roentgenogram; complete blood cell count (cbc); serum bilirubin, alkaline phosphatase, glutamic oxaloacetic transaminase (SGOT), creatinine phosphokinase (CPK), blood urea nitrogen, and glucose determinations; urinalysis; and electrocardiogram (ECG). Patients with advanced organic heart disease or with major ECG abnormalities (eg, ectopic arrhythmias, partial or complete block) were excluded from the study. The ECG, SGOT, and CPK determinations were repeated during and at the end of each treatment period. Patients were followed at frequent intervals as inpatients or outpatients for evaluation of toxic effects and response to therapy.

The therapeutic regimen consisted of subcutaneous (sc) injections of emetine at a dose level of either 1 mg/kg of body weight given daily for 10 days (not exceeding 65 mg/day) or 1.5 mg/kg of body weight given twice weekly for 5 weeks, on an alternate basis within each diagnostic group. Each patient received two courses of therapy which were separated by a 3–4-week rest period unless rapid progression of the disease, severe cardiac, neuromuscular, or gastrointestinal toxic effects, or death occurred prior to the second course of treatment.

The results of chemotherapy were evaluated on the basis of objective tumor regression using the following criteria. Complete response—complete disappearance of all measurable disease parameters; partial response, good—regression of measurable tumors by 50% or more; partial response, fair—regression of measurable tumors by 25%–50%; no response—progression of the disease, no regression of the tumor, or a response less than 25%.

RESULTS

Fifty-one patients were treated with emetine, of whom 28 received daily sc injections and 23 received twice weekly sc injections. The majority of the patients (36) had two courses of treatment; 15 patients received only one course because of obvious rapid progression of the disease. In four other patients treatment had to be stopped because of supraventricular tachycardia, progressive neuromyositis, necrotic skin lesions at the site of injection, and unrelated death, respectively, during the first treatment.

There were 29 men (median age 60 years, range 39–77 years) and 22 women (median age 58 years, range 26–82 years) in the study. Bronchogenic carcinoma comprised the largest group of tumors studied (16 patients). The remaining tumor types were more or less equally divided between cancers of the urinary, gastrointestinal, and female genital systems, and the breast (table 1). Prior therapy included surgical treatment with intent to cure in ten patients; palliative radiation therapy, chemotherapy, or hormonal therapy in 25 patients; and diagnostic explorations or biopsies only in 16 patients.

The number of patients who received daily or twice weekly emetine injections was approximately equal within each major cancer group. Thirty-two patients received two courses of therapy with the total emetine dose being 20 and 30 mg/kg, respectively, within the two treatment regimens.

There was either no measurable tumor response or progression of the disease in all patients studied.

Both treatment schedules were generally well tolerated. Mild pain at the injection site was present in 40 of 51 patients treated. Treatment was discontinued in only two of these patients: in one patient after the first five daily injections, and in the second patient at the end of the first twice weekly course. Necrosis of the skin at the injection sites developed in both patients. In 14 patients, marked pain at the injection sites and slight elevation of CPK levels were the only side effects of the treatment (table 2). In a small group of patients only asymptomatic elevations of CPK levels were observed. While gastrointestinal symp-

TABLE 1.—Phase II study of emetine in the treatment of 51 patients with solid tumors

Type of malignancy	No. of patients*	Treatment schedule†	
		Daily	Twice weekly
Bronchogenic carcinoma			
Epidermoid	9	5	4
Adenocarcinoma	4	2	2
Anaplastic	3	1	2
	16 (5)		
Genitourinary carcinoma			
Kidney, clear cell	6	3	3
Kidney, squamous	1	1	—
Prostate	2	1	1
	9 (3)		
Gastrointestinal carcinoma			
Stomach	3	2	1
Colon	2	—	2
Rectum	1	1	—
Pancreas	2	1	1
	8 (3)		
Breast carcinoma			
Ductal	4	2	2
Adenocarcinoma	2	1	1
	6 (2)		
Gynecologic carcinoma			
Ovarian	2	1	1
Endometrial	3	2	1
Cervix	1	—	1
	6 (3)		
Miscellaneous			
Kaposi's sarcoma	2	1	1
Melanoma	1	1	—
Thyroid, follicular	1	1	—
Esophagus, epidermoid	1	1	—
Tongue, epidermoid	1	1	—
	6 (3)		

* Figures in parentheses indicate the number of patients who received only one course of treatment.

† Number of patients treated.

toms were frequent in patients who received daily injections, neuromuscular and cardiac toxicity most often occurred in patients after twice weekly injections for 5 weeks and often after the completion of the second course of treatment. The neuromuscular and cardiac toxic effects were often preceded by elevated CPK values. The major cardiac toxic effect consisted of rapid atrial fibrillation in one patient and supraventricular tachycardia in three other patients. Each of these patients converted to normal sinus rhythm by either carotid sinus compression or digitalization. No hypertension or anginal pain was noted.

The elevated CPK values and neuromuscular toxic effects (generalized muscle weakness without sensory changes or muscle tenderness) persisted 2–4 weeks (average, 3 weeks) before

TABLE 2.—Toxic effects of emetine given sc to 51 patients with advanced cancers

Toxic effects	No. of patients (%)	CPK values *	
		Daily treatment †	Twice weekly treatment †
Marked local pain only	14 (28)	57/33–125 8 patients	72/25–158 6 patients
High serum CPK only	7 (14)	88/61–159 5 patients	166/78–254 2 patients
Nausea, vomiting	6 (12)	Normal 3 patients	Normal 3 patients
Diarrhea	1 (2)	Normal 1 patient	—
Neuromuscular	5 (9.8)	Normal 1 patient	155/30–261 4 patients
Cardiac arrhythmia	4 (7.8)	100 1 patient	90/10–177 3 patients
ST–T changes	4 (7.8)	Normal 2 patients	Normal 2 patients
Skin necrosis	2 (5.8)	85 1 patient	261 1 patient
Total	43	— 22 patients	— 21 patients

* In our laboratory, normal CPK values are 5–35 IU for women and 5–30 IU for men.
† Mean/range; serum CPK activity measured in IU/liter.

returning to normal. Minor transient T-wave changes alone on ECGs were observed in an additional four patients at the end of the treatment period.

There was no evidence of renal, hepatic, or hematologic toxic effects.

DISCUSSION

The earliest report of the use of emetine as an antineoplastic agent was by Lewisohn (4), in which he concluded that the antineoplastic action of emetine is purely caustic. This effect was observed when the drug was injected into the tumor, but there was no effect when it was given intravenously (iv). A year later, Van Hoosen (5) reported on 35 of 100 selected patients that she had treated with emetine. Later, she summarized her experiences in another report (11). The dose level and route and schedule of administration were variable from 130 mg given sc several times a day to 585 mg given iv once a week. The total dose was probably in the range of 12–22 mg/kg of body weight assuming the patients (all women) weighed about 50 kg each. There seem to have been only four patients who experienced complete remissions (three with breast carcinoma, one with lymphosarcoma, and one with labial carcinoma) although she reported that ten patients were cured and 11 improved out of 94 patients with advanced inoperable cancer. She commented that the patients that benefited most from the emetine treatment were those who went into temporary shock due to the toxic effects of emetine (11).

The interest in emetine as an antineoplastic agent was revived after animal experiments indicated its effectiveness in prolonging the life of rats with Yoshida sarcoma and mice with L1210 and P388 leukemias (7,12).

Complete remission of quadriplegia and pseudobulbar paralysis in a patient with glioma was reported after iv injections of 30–60 mg of emetine were given for a total dose of 1 g (10). One of the derivatives of emetine, dehy-

droemetine,[6] an effective amebicide with less toxicity (13,14), was reported to have induced complete and partial remissions in one patient with aleukemic myelogenous leukemia, one with adenocarcinoma of the rectum, and three with Hodgkin's disease (8,15). Further studies indicated that dehydroemetine may have also induced regression of carcinomas of the bladder, the prostate, and the tongue (16). However, the criteria of response and followup information were incomplete.

In a most recent phase I study by Panettiere and Coltman (9) emetine given iv at a dose level of 1.5 mg/kg of body weight weekly or every 4 days for a maximum total dose of 15 mg/kg of body weight, induced partial objective remissions in two patients with lung cancer and stopped progression of the disease in two other patients (one with carcinoma of the trachea and the other with carcinoma of the thyroid gland) (9).

In our study we used two dose schedules: one was similar to the regimen of Panettiere and Coltman but the drug was given sc; the other schedule which was usually used in the treatment of amebiasis consisted of ten daily injections. The toxic effects encountered with these regimens were less marked in comparison to those previously reported (9,17). The local pain of minor degree which was noted in almost all patients was moderate to marked in only 14 patients and resulted in discontinuance of the treatment in only one patient. The incidence of neuromuscular and cardiac toxicity was greater in patients who received 1.5 mg/kg of body weight twice weekly and often occurred at the end of the second course indicating a cumulative toxicity from a 50% higher dose of emetine rather than from schedule dependency.

The cardiac and neuromuscular toxic effects were accompanied by high serum CPK values in most patients; these values returned to normal within 2–3 weeks. The increased CPK values in patients with moderate to severe local pain or skin necrosis suggested that this increase may have been due to local irritation of the skeletal muscle at the emetine injection site rather than to release from cardiac muscle (table 2). The SGOT values remained within baseline levels in all patients. It would be of interest to measure the serum CPK levels in patients who received emetine iv in an attempt to detect impending cardiac toxic effects. In our patients, increasing serum CPK levels preceded cardiac arrhythmias in three of four patients.

None of the patients with high serum CPK levels were receiving other injections, psychotropic drugs, or phenothiazines that are known to increase serum CPK levels (18,19).

Contrary to previous reports, we observed no complete or partial regressions of the various solid tumors in our group of 51 patients, 32 of whom received two courses of treatment which totaled 20–30 mg/kg depending on the regimen studied. The arrest of the tumor growth or spread was not considered an adequate criteria for the antineoplastic effect of emetine. The total doses used in our study were similar to or even higher than those previously utilized. The use of dehydroemetine (8,16) or the iv route of administration (9), as well as differences in criteria for tumor response, may account for the disparity of results. Further studies may be indicated using emetine given iv as well as dehydroemetine which may be administered at higher doses because of lower toxicity. However, the sc injection of emetine at the dose levels and schedules used in this study has proved to be ineffective in treating various solid tumors.

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Methotrexate (NSC-740) and Bleomycin (NSC-125066) in the Treatment of Advanced Epidermoid Carcinoma of the Uterine Cervix [1,2,3]

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SUMMARY

The therapeutic effects of methotrexate and bleomycin in patients with locally advanced or metastatic carcinoma of the cervix were tested in a prospective randomized study. In 46 evaluable patients, the overall response rate was 25% for methotrexate and 26% for bleomycin. Thirteen percent of the patients treated with methotrexate and 13% of those treated with bleomycin had tumor regressions of more than 50%. There was no significant difference in the quality of response to the two drugs administered intravenously either as primary or as secondary therapy. Only one of 18 responsive patients had a complete remission. The median duration of response was 3 months (range, 1.5–6.5 months) for both drugs. One year from the start of chemotherapy there was no difference between the survival of the group of responding patients and that of the group of nonresponding patients. The most significant side effects were liver toxicity after methotrexate treatment (16%) and pulmonary toxicity after bleomycin treatment (9%). No cross resistance was observed.

[Cancer Chemother Rep 57:429–435, 1973]

The therapeutic effects of systemic chemotherapy in large series of patients with locally advanced or metastatic carcinoma of the uterine cervix are seldom reported. Conventional single-agent chemotherapy with cyclophosphamide, methotrexate, or vincristine produces a response rate of about 20% (1).[6] A higher percent of regression with cyclophosphamide plus vincristine in a limited series of patients was recently reported by Eagan et al (2). Bleomycin, a new antitumor antibiotic isolated in Japan from *Streptomyces verticillus*, was found to be effective against epidermoid carcinomas (3,4). In particular, this compound was reported in several studies using different dose schedules (3–6) to be effective in 20%–30% of patients with carcinoma of the cervix. No regression was seen, however, in the group of

[1] Received Apr 13, 1973; revised July 12, 1973; accepted July 19, 1973.

[2] Methotrexate: CAS reg. No. 59-05-2; glutamic acid, *N*-[*p*-[[(2,4-diamino-6-pteridinyl)methyl]methylamino]-benzoyl]-.

Bleomycin was supplied by Cancer Therapy Evaluation, Division of Cancer Treatment, National Cancer Institute.

[3] Presented at the 64th annual meeting of the American Association for Cancer Research, Atlantic City, Apr 11–13, 1973.

[4] National Cancer Institute, Milan, Italy.

[5] *Reprint requests to:* Dr. Gianni Bonadonna, National Cancer Institute, Via Venezian 1, Milan 20133, Italy.

[6] Cyclophosphamide: NSC-26271; CAS reg. No. 6055-19-2; 2*H*-1,3,2-oxazaphosphorine, 2-[bis(2-chloroethyl)-amino]tetrahydro-, 2-oxide, monohydrate.

Vincristine: NSC-67574; CAS reg. No. 2068-78-2; leurocristine, sulfate (1:1).

11 patients treated at Memorial Center (New York, NY) (7). The median duration of response was almost always in the range of 2–3 months.

The aims of the present study were (*a*) to compare through a controlled trial the therapeutic effects of methotrexate and bleomycin administered intravenously (iv) weekly and (*b*) to determine whether cross resistance develops in patients treated with the drugs.

PATIENTS AND METHODS

Patient Selection

From March 1972 to January 1973 a total of 64 patients with biopsy-proved carcinoma of the uterine cervix were entered into the study. Their mean age was 54 years (range, 36–70 years). The criteria for eligibility were (*a*) persistent or recurrent neoplasia within the pelvis after radical treatment and (*b*) distant metastases. Patients who were more than 70 years old and patients who had either a performance status lower than 60, no adequate objective tumor parameters, impaired kidney and/or liver function, or limited pulmonary reserve were excluded from this trial.

Sixty-three of the patients had been treated prior to the study (radical surgical treatment, four patients; radiation therapy [radium and/or cobalt 60], 47 patients; surgical treatment plus radiotherapy, four patients; surgical treatment plus chemotherapy, one patient; radiotherapy plus chemotherapy, six patients; chemotherapy alone, one patient). One patient had not been treated previously. All eight patients that had received chemotherapy had been treated with cyclophosphamide. The mean interval between initial treatment and chemotherapy with methotrexate or bleomycin was 21 months (range, 0.5–195 months).

Clinical Evaluation

The following pretreatment tests were performed: pelvic examination including bidimensional tumor measurements whenever possible; X-ray films of the pelvic bones and of the chest; iv urography; foot lymphography (in 40 of the 64 patients); pelvic arteriogram (in two patients who had intrapelvic disease but no abnormal displacement of the ureters after excretory urogram); complete blood cell count (including platelet count); urinalysis; determination of values for blood urea nitrogen (BUN) and serum creatinine, uric acid, electrolytes (including calcium and phosphorus), bilirubin, alkaline phosphatase, total proteins, glutamic oxaloacetic transaminase (SGOT), and glutamic pyruvic transaminase (SGPT). The evaluation during study included the following: tumor measurements (physical and roentgenologic examination) about once a month and before crossover treatment; blood cell counts once a week before each dose of methotrexate; determination of blood chemical values every 6 weeks or as indicated; and chest X-ray films every 3–4 weeks during treatment with bleomycin.

Drug Administration

Patients were almost always treated on an outpatient basis. The drugs and dose schedules are outlined in table 1. Patients entering into the study were randomly assigned to initial treatment with either methotrexate or bleomycin for 6 weeks. The crossover was carried out when after 3 weeks from the sixth dose no appreciable tumor regression was observed (induction failure) or at any time in the presence of progressive disease or prohibitive toxicity (ie, renal or hepatic damage attributable to methotrexate, severe myelosuppression [a leukocyte count less than 2000 cells/mm^3 and/or a platelet count less than 50,000 platelets/mm^3] for more than 3 weeks from the last dose of methotrexate, or radiologic evidence of interstitial lung lesions attributable to bleomycin). In responsive patients, maintenance treatment was given with the same drug and dose, but the treatment was administered every 2 weeks. The dose-reduction schedule that was adopted in the presence of methotrexate-induced myelosuppression is shown in table 2. No other form of antitumor therapy was allowed during the period of study.

Criteria of Drug Response

Whenever possible, all tumors were measured in at least the two longest diameters and measurements were recorded in centimeters. Complete remission was defined as the disappear-

TABLE 1.—Design of study*

Random assignment to—		Maintenance treatment in responsive patients		
Drug	Dose	Drug	Dose	Crossover treatment for:
Methotrexate	40 mg/m²/wk × 6, iv	Methotrexate	40 mg/m² every 2 wks, iv	Induction failure, progression of disease, prohibitive toxicity
	or			
Bleomycin	30 mg/m²/wk × 6, iv	Bleomycin	30 mg/m² every 2 wks, iv	Induction failure, progression of disease, prohibitive toxicity

* *See* text for further explanation.

TABLE 2.—Dose-reduction schedule in the presence of myelosuppression secondary to methotrexate treatment

Wbc (cells/mm³)	Platelet count (platelets/mm³)	Next dose (% of initial dose)
>4000	>150,000	100
2500–3999	100,000–149,000	50
2000–2499	50,000–99,000	25
<2000	<50,000	No drug given for 1 wk

ance of all clinical and radiologic evidence of active tumor for a minimum of 4 weeks. Partial remission was defined as a 50% or greater decrease in the products of the longest tumor diameters for a minimum of 4 weeks without a simultaneous increase in the size of any lesion or the appearance of new lesions. Objective improvement was defined as a 25%–50% decrease in measurable tumor lesions for at least 1 month in the absence of progressive disease elsewhere. Progressive disease was defined as an unequivocal increase of more than 25% in the size of any measurable lesion or the appearance of new lesions.

RESULTS

The characteristics of the patients studied are summarized in table 3. Of the 64 patients entered into the trial, 46 are presently evaluable for response to primary therapy with methotrexate or bleomycin, and 25 are evaluable for response to secondary therapy. Eighteen patients were not considered evaluable for a number of reasons (*see* table 3).

Response to therapy is reported in table 4. It appears that both methotrexate and bleomycin yield approximately the same overall results when given as primary treatment as when given as secondary treatment. Complete remission was observed in only one of 18 responsive patients, whereas partial remissions were almost equally divided between the patients with 25%–50% reductions in tumor size and patients with greater than 50% reductions in tumor size. Analysis of the median durations of response also failed to show a difference between methotrexate and bleomycin (table 5). The actuarial analysis of survival from the start of chemotherapy shows no significant difference between the group of responsive patients and the group of nonresponsive patients. In fact, 1 year after the beginning of the study 65% of the 18 responding patients and 55% of the 28 nonresponding patients are alive.

Cross resistance did not occur. Although two patients responsive to methotrexate and two patients responsive to bleomycin failed to respond subsequently to the crossover drug, two patients showing induction failure after

TABLE 3.—Characteristics of patients entered into study*

	No. of patients—		
	Total	Treated with methotrexate	Treated with bleomycin
Entered into study	64		
Evaluable for response to primary therapy	46	23	23
Evaluable for response to secondary therapy	25	9	16
Not evaluable	18		
Lack of adequate parameters	3		
Rapidly progressing cancer	3		
Lost to followup	10		
Early severe toxicity	2		
Still being treated	13	7	6

* As of Apr 1, 1973.

TABLE 4.—Response to primary and secondary therapy

Therapy	Drug	No. of evaluable cases	Type of response				Overall response rate (%)
			None	25%–50% regression	>50% regression	Complete remission	
Primary	Methotrexate	23	16	4	2	1	7/23 (30)
	Bleomycin	23	16	4	3	—	7/23 (30)
Secondary	Methotrexate	9	8	—	1	—	1/9 (11)
	Bleomycin	16	13	1	2	—	3/16 (19)
Primary plus secondary	Methotrexate	32	24	4	3	1	8/32 (25)
	Bleomycin	39	29	5	5	—	10/39 (26)

TABLE 5.—Median duration of regression of disease in 18 responsive patients

Therapy	Drug	No. of responsive patients	Duration of response (mos)	
			Median	Range
Primary	Methotrexate	7	3	1.5–6.5
	Bleomycin	7	3	1.5–3.5
Secondary	Methotrexate	1	3	—
	Bleomycin	3	3	1.5–5.0

primary treatment with methotrexate and one patient failing to respond to bleomycin did respond to secondary therapy with the cross-over drug. One patient was responding to both drugs.

After treatment with bleomycin and methotrexate, five patients were given adriamycin† (75 mg/m² every 3 weeks); subsequently, two of these patients had regressions greater than 50%.

† Adriamycin: NSC-123127.

The type of response in relation to the site of the metastasis is reported in table 6. Regressions were observed in all types of lesions, but they were particularly significant at the level of intrapelvic disease (overall response rate of 73%). Again, no significant difference between methotrexate and bleomycin was noted (regressions occurred in nine of 19 patients and in ten of 19 patients, respectively, at the level of intrapelvic disease). About one half of the patients with pelvic disease also had vaginal recurrence.

The manifestations of toxicity for both drugs are listed in table 7. Bone marrow suppression occurred in 22% of the patients, and only one patient had leukopenia with a wbc less than 1000 cells/mm³ and a platelet count less than 50,000 platelets/mm³. Myelosuppression occurred only after the administration of methotrexate. The incidence of stomatitis, gastrointestinal disturbances (nausea, vomiting, diarrhea), and loss of hair was higher after treatment with methotrexate than after treatment with bleomycin. Liver toxicity with increased bromsulphalein retention and elevated alkaline phosphatase, SGOT, and SGPT values was observed in 16% of the patients given methotrexate. Abnormal levels of bilirubin were noted in only one patient. The clinical diagnosis of hepatic damage was not confirmed through liver biopsy. The damage occurred after the administration of a mean dose of 140 mg/m² (range, 40–240 mg/m²) and, with one exception, was always reversible within 4–6 weeks after the discontinuation of treatment. In two patients treated with methotrexate, transient increases in BUN, serum creatinine, and serum potassium were noted. These changes might be compatible with renal toxicity (8). The classic roentgenologic signs of early pulmonary toxicity (9) were observed in four (9%) of the patients treated with bleomycin after a mean dose of 60 mg/m² (range, 45–180 mg/m²). Upon prompt discontinuation of treatment with the drug, lung lesions were reversible within 6–10 weeks. In nine patients, crepitant rales were heard at one or both bases of the lungs. Initial pulmonary damage was also suspected even in the absence of abnormalities in X-ray films (9). In 65% of the patients given bleomycin, the typical cutaneous stria were observed especially throughout the back.

One patient died at home because of drug toxicity. About 1 week after the first dose of methotrexate she had severe stomatitis and diarrhea, followed by progressive jaundice and marrow aplasia with hemorrhage and infection. Before starting therapy her blood chemical values and excretory urogram had all appeared to be within normal limits.

During primary treatment patients received 68% of the projected dose of methotrexate and 83% of the projected dose of bleomycin. During secondary therapy these figures were 50% and 84%, respectively.

COMMENT

The preliminary results of this trial show that both methotrexate and bleomycin exert a

TABLE 6.—Regression of disease in relation to site of metastasis

Site of metastasis	No. of patients	Type of response			
		None	25%–50% regression	>50% regression	Complete remission
Intrapelvic	26	7	13	5	1
Retroperitoneal nodes	9	6	3	—	—
Peripheral nodes	2	1	1	—	—
Lung	2	—	1	1	—
Bone	2	1	—	—	1
Liver	1	—	—	—	1
Leg edema	6	2	—	3	1

TABLE 7.—Manifestations of toxicity in 64 patients

Toxic effect	No. of patients with toxic effect (%)	
	Methotrexate (total treated, 51 patients)	Bleomycin (total treated, 46 patients)
Myelosuppression *	11 (22)	None
Stomatitis	14 (27)	6 (13)
Gastrointestinal disturbances	30 (59)	20 (43)
Loss of hair	19 (37)	13 (28)
Liver toxicity	8 (16)	None
Lung toxicity	None	4† (9)
Skin toxicity	None	30 (65)

* Wbc <3000 cells/mm³ or platelet count <130,000 platelets/mm³ or both.

† Confirmed by chest X-ray film.

therapeutic effect on carcinoma of the uterine cervix. Neither drug appears to be superior to the other for either primary or secondary therapy. The quality and median duration of the regressions observed fail to show that a consistent therapeutic control of this disease was achieved with the dose schedule used in this study. This observation is also clearly reflected by the survival curves of responsive and nonresponsive patients. Therefore, on the basis of these data one can conclude that both methotrexate and bleomycin have a limited therapeutic effect against squamous cell carcinoma of the cervix.

However, since the available data indicate that carcinoma of the cervix is not particularly susceptible to single-agent chemotherapy, one can look at our data from a different angle. An increase in the dosage of both methotrexate and bleomycin would probably result in a higher incidence of toxicity rather than a higher response rate. Therefore, since all drugs tested so far in this type of cancer, including adriamycin (10,11) and hexamethylmelamine (12),[8] yield a 20%–30% regression rate, the next logical step would be to design appropriate controlled trials of combination chemotherapy. If more than one combination will produce a response rate of more than 30%, then sequential combination chemotherapy can be tried in the attempt to achieve a better control of this disease.

[8] Hexamethylmelamine: NSC-13875; CAS reg. No. 645-05-6.

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Combination Chemotherapy With Adriamycin (NSC-123127) and 1-(2-Chloroethyl)-3-cyclohexyl-1-nitrosourea (CCNU; NSC-79037) [1,2,3]

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SUMMARY

Forty-one patients with various solid tumors were treated with a combination of adriamycin and 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea (CCNU). Adriamycin was given every 3 weeks at a dose ranging from 40 to 75 mg/m², and CCNU was given every 6 weeks at a dose ranging from 65 to 125 mg/m². The cases of 33 of the 41 patients were considered evaluable. Three patients had complete responses and 11 had partial (greater than 50%) remissions for a response rate of 34% considering all patients treated or 42% considering only those with evaluable cases. Responses were noted in patients with cancer of the breast (eight of 13), squamous cell carcinoma of the head and neck (three of six), malignant melanoma (one of eight), undifferentiated carcinoma with unknown primary site (one of five), and lymphoma (one of four). The combination was well tolerated; its main toxic effects were vomiting, alopecia, and hematologic depression. With this combination there were two nadirs of myelosuppression: one occurred on Day 15 and the other occurred on Day 36. Prolonged thrombocytopenia was occasionally seen after multiple doses of CCNU.

[Cancer Chemother Rep 57:437–445, 1973]

Adriamycin, a new antitumor antibiotic produced by the mold *Streptomyces peucetius* var *caesius*, is a 14-hydroxylated derivative of daunomycin. Like its parent compound, adriamycin is thought to intercalate with DNA and thus prevent cell division (1). In clinical trials, the main toxic effects of adriamycin have been alopecia, stomatitis, cardiotoxicity, and myelosuppression (principally granulocytopenia), which is maximal approximately 2 weeks after a single dose (2,3). The drug has shown activity against a wide variety of cancers including leukemia (4), lymphoma (5), cancer of the breast (6), sarcoma (2,7), carcinoma of the bladder (3), and testicular tumors (8).

One major drawback with adriamycin has

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[2] Supported by Public Health Service grants CA-10379 and CA-03754 from the National Cancer Institute, National Institutes of Health, Department of Health, Education, and Welfare.

[3] CCNU and adriamycin were supplied by Cancer Therapy Evaluation, Division of Cancer Treatment, NCI. Chemical nomenclature, NSC numbers, and CAS registry numbers for all compounds mentioned in this paper are listed in the List of Compounds at the end of the text.

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been its inability to cross the blood-brain barrier and achieve a measurable concentration in the cerebrospinal fluid (9). In our earlier studies with adriamycin alone, we observed the development of metastases in the central nervous system (CNS) (especially in patients with carcinoma of breast) despite the continued regression of the disease at other sites of metastasis.

1-(2-Chloroethyl)-3-cyclohexyl-1-nitrosourea (CCNU), one of the new nitrosoureas for oral administration, is highly soluble in lipids and therefore has the ability to penetrate the blood-brain barrier (10). CCNU has been reported to be active against a number of human brain tumors (11). It has also been shown to be effective against Hodgkin's disease, non-Hodgkin's lymphoma, and, to a lesser extent, cancer of the breast and squamous cell carcinoma (12,13). The main toxic effects of CCNU are nausea and vomiting on the day of administration and delayed hematologic toxicity which is maximal 4–5 weeks after administration.

Since myelosuppression, the major dose-limiting toxic effect of both adriamycin and CCNU, appeared to have a different time course with each agent and since the other toxic effects of the two drugs did not appear to overlap, combination therapy with these two agents was thought to be feasible. Additional studies by Schabel had shown that although neither drug given alone 48 hours before the median day of death appeared to be especially effective against advanced murine L1210 leukemia implanted intraperitoneally (10^7 cells), definite therapeutic potentiation occurred with simultaneous therapy (Schabel, F.M., Jr., personal communication). This study was designed to determine the efficacy and toxicity of combination treatment with adriamycin and CCNU in patients with advanced solid tumors, especially cancer of the breast.

MATERIALS AND METHODS

Patients

Forty-one patients with a variety of solid tumors were treated with a combination of adriamycin and CCNU from December 1971 through March 1973. There were 27 women and 14 men. Their ages ranged from 20 years to 74 years; the median age was 47 years. Patient characteristics and disease diagnoses are indicated in table 1. None of the patients had received daunomycin, adriamycin, or a nitrosourea prior to this study.

Drug Regimen

The initial starting dose for CCNU was 65 mg/m² given orally every 6 weeks starting on Day 1 (or approximately 50% of the recommended starting dose for single drugs [12]); the initial starting dose for adriamycin was 40 mg/m² given intravenously (iv) every 3 weeks (again, approximately 50% of the recommended starting dose for single drugs [3]). After initial observations had indicated that this dose was well tolerated with minimal myelosuppression occurring, treatment for subsequent patients was started at a dose of 100 mg/m² of CCNU every 6 weeks and 60 mg/m² of adriamycin every 3 weeks. Observations with this regimen indicated that the hematopoietic suppression occurring in the second 3 weeks of each course was prolonged (presumably because of the overlapping toxicity of the two agents). Consequently, from July 1972 on all patients were treated as follows: on Day 1, 100 mg/m² of CCNU was administered orally and 60 mg/m² of adriamycin was administered iv; on Day 22, 45 mg/m² of adriamycin was administered iv. The second course of combination therapy was started 6 weeks after Day 1 (Day 43). The total dose of adriamycin was limited to 550 mg/m² to avoid the development of cardiomyopathy (14). After this, treatment was continued with CCNU alone. A provision of the protocol permitted the raising or lowering of doses of both agents after the first course to ensure an adequate but safe level of hematopoietic suppression. An additional provision permitted the omission of adriamycin treatment on Day 22 if the white blood cell count (wbc) was 2000 cells/mm³ (this applied to only two patients).

Adriamycin was supplied in 10-mg vials. The drug was dissolved in 50–100 ml of 5% dextrose and water and given as a 15–30-minute iv infusion with care being taken to avoid extravasation. CCNU was supplied as 40- and 100-mg capsules which were kept

TABLE 1.—Data on patients, prior therapy, and response to therapy with adriamycin plus CCNU

Diagnosis	Patient No.	Age, sex	Metastatic involvement	Prior therapy	Starting dose* (mg/m²)		Response†	Duration of response (mos)
					Adriamycin (Day 1/ Day 22)	CCNU (Day 1)		
Carcinoma of the breast	1	49,F	Lungs, bone, soft tissue	Oophorectomy, radiotherapy	60/45	100	CR	2+
	2	50,F	Ascites and pleural effusions, bone, bone marrow	5-FU	60/45	100	PR	1+
	3	43,F	Bone	Oophorectomy, androgen	60/45	100	NC	—
	4	62,F	Bone, nodes	None	60/45	100	NC	—
	5	38,F	Nodes	Oophorectomy	60/60	100	CR	6
	6	57,F	Lungs	5-FU + cyclophosphamide	40/40	65	PR	5+
	7	60,F	Bone, liver, bone marrow	None	40/40	65	NC	—
	8	66,F	Lungs, skin, nodes	Radiotherapy, estrogen	40/40	65	PR	8
	9	55,F	Chest wall, nodes	Oophorectomy, radiotherapy, androgen	—	—	PV	—
	10	48,F	Soft tissue, nodes	Radiotherapy, 5-FU, cyclophosphamide, vincristine	40/40	65	NC	—
	11	55,F	Subcutaneous nodules, lungs, nodes	5-FU, cyclophosphamide, methotrexate, vincristine	60/45	100	PR	5+
	12	53,F	Skin, nodes, bone, bone marrow	None	60/45	100	PR	2+
	13	30,F	Liver, nodes, bone, bone marrow	Oophorectomy	60/45	100	PR	2+
Melanoma	14	20,F	Nodes, abdominal viscera	NSC-45388 + procarbazine, hydroxyurea	40/40	65	PD	—
	15	31,M	Lungs, skin, nodes, liver, spleen	NSC-45388 + procarbazine, 5-azacytidine	40/40	65	NC	—
	16	40,F	Lungs, bone, brain, nodes	NSC-45388 + procarbazine	40/40	65	ED	—
	17	39,M	Lungs	NSC-45388 + procarbazine	40/40	65	PD	—
	18	21,M	Lungs, subcutaneous nodules	NSC-45388 + procarbazine	40/40	65	PR	1
	19	41,F	Subcutaneous nodules	NSC-45388 + procarbazine	40/40	65	PD	—
	20	25,M	Lungs, subcutaneous nodules, nodes	NSC-45388 + procarbazine	40/40	65	PD	—
	21	66,F	Liver	NSC-45388 + procarbazine	40/40	65	PD	—

TABLE 1.—Data on patients, prior therapy, and response to therapy with adriamycin plus CCNU—Continued

Diagnosis	Patient No.	Age, sex	Metastatic involvement	Prior therapy	Starting dose* (mg/m²) Adriamycin (Day 1/ Day 22)	Starting dose* (mg/m²) CCNU (Day 1)	Response†	Duration of response (mos)
Squamous cell carcinoma of the head and neck	22 (Maxillary sinus)	61,M	Local extension	None	60/45	100	PD	—
	23 (Tonsil)	54,M	Local extension, cervical nodes	Hydroxyurea + radiotherapy	60/60	100	PR	1
	24 (Naso-pharynx)	42,M	Local extension	Methotrexate, prednisone, bleomycin, vincristine	60/60	100	PR	2
	25 (Larynx)	69,M	Liver, lungs, abdominal wall	None	40/40	65	ED	—
	26 (Naso-pharynx)	31,M	Nodes, local extension	Bleomycin, methotrexate	40/40	65	PR	1.5
	27 (Tonsil)	46,M	Cervical nodes	Bleomycin, vincristine	60/60	100	ED	—
Undifferentiated carcinoma with unknown primary site	28	61,F	Brain, liver, subcutaneous nodules	5-FU	60/60	100	ED	—
	29	38,F	Abdominal viscera	None	40/40	65	PD	—
	30	34,F	Bone	None	60/45	100	PD	—
	31	46,M	Bone, lungs	None	60/45	100	PD	—
	32	47,F	Brain, lungs	None	60/45	100	PR	4+
Lymphoma	33 (Nodular, poorly differentiated lymphocytic lymphoma)	45,F	Lymph nodes, spleen	Chlorambucil, vincristine + prednisone	60/60	100	PD	—
	34 (Nodular, poorly differentiated lymphocytic lymphoma)	29,M	Subcutaneous nodules	Radiotherapy, mechlorethamine, prednisone, procarbazine, vincristine	60/45	100	CR	2+
	35 (Histiocytic lymphoma)	74,F	Lymph nodes, bone, liver	Cyclophosphamide, vincristine, cytosine arabinoside + prednisone	40/40	65	ED	—
	36 (Hodgkin's disease)	21,F	Lymph nodes	Mechlorethamine, vincristine, procarbazine, prednisone (MOPP); vinblastine + bleomycin	—	—	PV	—
Renal cell carcinoma	37	56,M	Lungs, bones	None	40/40	65	PD	—
	38	58,M	Liver, right flank mass	Progesterone	40/40	65	NC	—

TABLE 1.—Data on patients, prior therapy, and response to therapy with adriamycin plus CCNU—Continued

Diagnosis	Patient No.	Age, sex	Metastatic involvement	Prior therapy	Starting dose* (mg/m²) Adriamycin (Day 1/ Day 22)	Starting dose* (mg/m²) CCNU (Day 1)	Response†	Duration of response (mos)
Miscellaneous	39 (Ovarian carcinoma)	63,F	Lymph nodes, pelvis, chest	5-FU, cyclophosphamide, melphalan	60/45	100	ED	—
	40 (Chemodectoma)	47,M	Bones	None	40/40	65	NC	—
	41 (Histiocytic lymphoma and undifferentiated adenocarcinoma)	57,F	Lymph nodes, liver, stomach	Cyclophosphamide, chlorambucil, prednisone	60/60	100	PD	—

*See text for further explanation of dosage.
†CR = complete remission; PR = partial response; NC = no change; PD = progressive disease; PV = protocol violation; ED = early death.

refrigerated in amber bottles until administration. The capsules were taken on an empty stomach.

Tumor size was determined by calculating the products of the greatest diameters of the tumor at right angles to each other. Partial response was defined as a 50% or greater reduction in the sum of these products lasting 1 month or longer. Complete remission was defined as the disappearance of all measurable disease and all symptoms for at least 1 month.

Blood cell counts, platelet counts, and differentials were determined weekly for all patients, and liver and renal function tests were performed twice monthly. Tumor measurements were recorded at least every 3 weeks, and appropriate radiologic studies were repeated every 6 weeks. Electrocardiograms were obtained prior to each dose of adriamycin.

RESULTS

Forty-one patients were treated with the combination of adriamycin and CCNU. The cases of eight patients were not considered evaluable (six patients died within the first 4 weeks of therapy from rapidly advancing malignant disease, and two patients had major protocol violations in which additional drugs were given and the dosage regimen outlined previously was not followed). Three complete remissions and 11 partial remissions were observed for an objective response rate of 42% if only the 33 patients with evaluable cases are considered or 34% if all patients registered for the study are considered. The median duration of response was 4+ months eight of the 14 responding patients are still in remission (this includes two of the three patients that had complete remissions). Responses occurred in patients with cancer of the breast, squamous cell carcinoma of the head and neck, malignant melanoma, undifferentiated carcinoma with unknown primary site, and malignant lymphoma (table 2). Seven additional patients had no progression of their disease while receiving therapy, including two patients with extensive bone metastases (patient No. 40 who had chemodectoma and patient No. 7 who had cancer of the breast). Both of the latter patients had had rapidly progressing disease prior to the start of therapy; both remain free from bone pain 15+ and 9+ months, respectively, after the start of treatment with this regimen.

Toxicity

The hematologic toxicity observed in the 41 patients treated is summarized in table 3. Myelosuppression consisted mainly of leukopenia with granulocytopenia. Thrombocytopenia was observed more commonly in those

TABLE 2.—Response to treatment with adriamycin plus CCNU

Diagnosis	No. of patients entered into study	No. of evaluable cases	No. of patients who had—			
			Complete remission	Partial remission	No change	Progression of disease
Carcinoma of the breast	13	12	2	6	4	0
Melanoma	8	7	0	1	1	5
Squamous cell carcinoma of the head and neck	6	4	0	3	0	1
Undifferentiated carcinoma with unknown primary site	5	4	0	1	0	3
Lymphoma	4	2	1	0	0	1
Renal carcinoma	2	2	0	0	1	1
Miscellaneous	3	2	0	0	1	1
Total	41	33	3	11	7	12

patients who were treated for three or more courses, and, in this setting, it was occasionally prolonged, forcing a delay (median delay, 11 days; range, 6–38 days) in the start of the next course for seven patients. There were two nadirs of leukopenia (one on Day 15 and the other on Day 36). Near complete recovery occurred on Day 22, and in all but the seven patients mentioned previously recovery was also seen by Day 43. All of the four patients who had dose escalations in the last phase of this study had unacceptable levels of granulocytopenia with three episodes of sepsis recorded (table 3).

Each patient had marked loss of scalp hair as a result of adriamycin treatment, but stomatitis was seen in only two patients. Nausea and vomiting occurred on Day 1 of each course in all patients and on Day 22 as well in about half of the patients. Two drug-related deaths were observed. Patient No. 39 died from septicemia (gram-negative organisms) associated with severe granulocytopenia 14 days after her first course of therapy. Patient No. 38 received a total adriamycin dose of 560 mg/m^2; this exceeded the recommended stopping point. Shortly after the patient had received his last dose, severe adriamycin-induced cardiomyopathy developed; it did not respond to supportive medical therapy. This was the only significant case of cardiomyopathy in this patient population.

Because responses were noted in three patients with far-advanced squamous cell carcinoma of the head and neck that previously had proved refractory to surgical treatment, radiotherapy, and chemotherapy, we think that the combination warrants further investigation in patients with this diagnosis. The response rate in patients with malignant melanoma was disappointing, with only one partial response occurring in the eight patients treated. However, all eight patients had failed to respond to intensive combination chemotherapy given prior to this trial. The number of patients in other diagnostic categories, with the possible exception of malignant lymphoma, is too small for comment. The cases of two patients with malignant lymphoma were evaluable; one patient with advanced disease had a complete response. Since both adriamycin and CCNU are effective as single agents against advanced malignant lymphoma, further trials of the combination in patients in this diagnostic category may be fruitful.

This study has shown that combination chemotherapy with adriamycin and CCNU is practical and perhaps especially rewarding in patients with diseases that have a high propensity for metastasis to the CNS. The results reported for patients with breast cancer or advanced squamous cell carcinoma of the head and neck are favorable; the results obtained in patients with malignant lymphoma indicate that further trials may be warranted.

TABLE 3.—Myelotoxicity in patients treated with adriamycin plus CCNU

Dose (mg/m²)			Median nadir counts × 10^3 (range) on—								
			Day 15			Day 22			Day 36		
Adriamycin (Day 1/Day 22)	CCNU (Day 1)	No. of courses	Wbc	Granulocytes	Platelets	Wbc	Granulocytes	Platelets	Wbc	Granulocytes	Platelets
40/40	65	22	4.2 (1.8–12.2)	2.5 (0.8–8.1)	> 250 (91–523)	5.5 (3.2–14.2)	4.1 (2.1–12.6)	240 (32–410)	6.0 (1.8–12)	4.0 (0.6–5.7)	230 (50–400)
60/60	100	14	2.8 (0.5–5.0)	1.4 (0.2–4.0)	> 250 (94–430)	5.9 (2.5–13.2)	4.3 (2.1–6.9)	180 (45–430)	2.7 (0.8–3.6)	2.2 (0.8–2.8)	> 250 (61–512)
60/45	100	20	2.9 (0.5–6.7)	1.8 (0.3–5.0)	120 (56–300)	3.8 (1.5–9.9)	3.2 (0.8–7.4)	198 (42–346)	3.7 (1.1–8.0)	2.1 (0.6–6.0)	> 250 (97–447)
75/60	125	4	2.4 (1.4–2.9)	0.2 (0.2–0.6)	> 250 (80–462)	4.9 (2.2–7.6)	3.2 (1.7–5.4)	> 250 (60–386)	1.6 (0.9–6.1)	0.8 (0.2–3.6)	> 250 (42–300)

Testing to see if the combination is superior to the individual agents will be another important goal of further investigations.

DISCUSSION

The regimen of combination chemotherapy with adriamycin and CCNU studied in this trial was for the most part well tolerated. The hematologic toxicity that occurred with CCNU administered orally on Day 1 at a dose of 100 mg/m² and adriamycin administered iv on Day 1 at a dose of 60 mg/m² and on Day 22 at a dose of 45 mg/m² and repetition of the entire cycle every 6 weeks was acceptable in the 13 patients (20 courses) studied. As the regimen indicates, it was necessary to lower the dose of adriamycin on Day 22 to compensate for the delayed myelosuppression from CCNU. There was minimal thrombocytopenia with this regimen; granulocytopenia was the main myelosuppressive effect observed. We had previously noted the platelet-sparing property of adriamycin in phase I studies with this drug alone (3). Prolonged thrombocytopenia did occur occasionally in patients who received three or more courses and was, therefore, thought to be related to the cumulative effects of CCNU. No incidents of hemorrhage occurred while patients were thrombocytopenic.

Encouraging results were obtained in patients with cancer of the breast and in patients with squamous cell carcinoma of the head and neck. Three of the four patients with cancer of the breast who had responded to prior chemotherapy had partial responses in this trial. Two of the eight patients who had not had previous drug therapy (including six with extensive involvement of bones or bone marrow or both) had complete remissions and four had partial remissions. None of the patients with cancer of the breast had initial progression of disease while being treated with this regimen. Although the number of patients in this study is too small for significant conclusions, the response rate compares favorably with a response rate of only 38% noted in a previous study of treatment of breast cancer with adriamycin alone in a somewhat more refractory population (6). One reason for studying combination therapy with adriamycin and CCNU was our observation that for five of the eight patients with carcinoma of the breast treated with adriamycin alone who had relapses, the cause of relapse was the development of metastases in the CNS (6). In only one of the eight patients with carcinoma of the breast responding in this study did CNS metastases develop. Although this preliminary trend is encouraging, it is obvious that further studies will be necessary before any conclusions can be reached regarding the possible prophylactic role of CCNU for brain metastases.

LIST OF COMPOUNDS

5-Azacytidine: NSC-102816; CAS reg. No. 320-67-2; *s*-triazin-2(1*H*)-one, 4-amino-1-β-D-ribofuranosyl-

Bleomycin: NSC-125066

CCNU: CAS reg. No. 13010-47-4

Chlorambucil: NSC-3088; CAS reg. No. 305-03-3; butyric acid, 4-[*p*-[bis(2-chloroethyl)amino]phenyl]-

Cyclophosphamide: NSC-26271; CAS reg. No. 6055-19-2; 2*H*-1,3,2-oxazaphosphorine, 2-[bis(2-chloroethyl)-amino]tetrahydro-, 2-oxide, monohydrate

Cytosine arabinoside: NSC-63878; CAS reg. No. 69-74-9; cytosine, 1-β-D-arabinofuranosyl-, monohydrochloride

Daunomycin: NSC-82151; CAS reg. No. 23541-50-6; daunorubicin (NSC-83142)

5-FU: NSC-19893; CAS reg. No. 51-21-8; 5-fluorouracil

Hydroxyurea: NSC-32065; CAS reg. No. 127-07-1

Mechlorethamine: NSC-762; CAS reg. No. 55-86-7; diethylamine, 2,2′-dichloro-*N*-methyl-, hydrochloride; HN2; nitrogen mustard

Melphalan: NSC-8806; CAS reg. No. 3223-07-2; alanine, 3-[*p*-[bis(2-chloroethyl)amino]phenyl]-, monohydrochloride, L-

Methotrexate: NSC-740; CAS reg. No. 59-05-2; glutamic acid, *N*-[*p*-[[(2,4-diamino-6-pteridinyl)methyl]-methylamino]benzoyl]-

NSC-45388: CAS reg. No. 4342-03-4; imidazole-4-carboxamide, 5-(3,3-dimethyl-1-triazeno)-

Prednisone: NSC-10023; CAS reg. No. 53-03-2; pregna-1,4-diene-3,11,20-trione, 17,21-dihydroxy-

Procarbazine: NSC-77213; CAS reg. No. 491-88-3; *p*-toluamide, *N*-isopropyl-α-(2-methylhydrazino)-, monohydrochloride

Progesterone: NSC-9704; CAS reg. No. 57-83-0; pregna-4-ene-3,20-dione

Vinblastine: NSC-49842; CAS reg. No. 6449-03-2; vincaleukoblastine, sulfate (1:1), monohydrate

Vincristine: NSC-67574; CAS reg. No. 2068-78-2; leurocristine, sulfate (1:1)

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Clinical Experience With a New Anticancer Agent, Carbazilquinone (NSC-134679) [1,2]

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SUMMARY

Carbazilquinone, an alkylating agent that is structurally similar to mitomycin C, was used in the treatment of 32 patients (41 courses) who had various malignant diseases. The drug was injected intravenously at a dose level of 0.02 or 0.04 mg/kg/day for as many days as possible. Thirty of 32 patients were evaluable and received carbazilquinone for the first time. According to the criteria for evaluation employed in our Department, the effects of the drug were evaluated as markedly effective in four of 30 patients (13.3%), effective in one patient (3.3%), unchanged in 13 patients (43.4%), and ineffective in 12 patients (40.0%). Leukopenia developed in 58.1% of the patients, thrombocytopenia in 22.6%, and anorexia in 16.1%. These represented the main toxic effects of the drug. Treatment was discontinued most frequently because of leukopenia. No side effects which involved liver or kidney functions were noted. Of the dose levels given, 0.02 mg/kg/day was superior to 0.04 mg/kg/day with respect to the clinical and side effects of the drug.

[Cancer Chemother Rep 57:447–457, 1973]

A new anticancer agent, carbazilquinone, which was synthesized and developed by Arakawa et al (Center Research Laboratories, Sankyo Co., Ltd., Japan) (1), is an alkylating agent; its structure is shown in figure 1. This structure is similar to those of mitomycin C and 2,3,5-tris(1-aziridinyl)-*p*-benzoquinone.[4]

Arakawa et al (1) have already studied the antitumor effects of carbazilquinone in experimental L1210 leukemia in mice, and they reported the superiority of this compound over methotrexate, mitomycin C, cyclophosphamide, and 2,3,5-tris(1-aziridinyl)-*p*-benzoquinone from the standpoint of its chemotherapeutic index and life-prolonging effects.[5] The clinical effects of this agent on human leukemia and other tumors of the hematopoietic system were presented at the Eighth General Meeting of the

[1] Received May 22, 1973; revised July 3, 1973, accepted July 30, 1973.

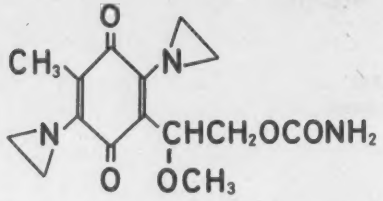
[2] Carbazilquinone: CAS reg. No. 24279-91-2; *p*-benzoquinone, 2,5-bis(1-aziridinyl)-3-(2-hydroxy-1-methoxyethyl)-6-methyl-, carbamate (ester).

[3] Department of Clinical Cancer Chemotherapy, Research Institute for Tuberculosis, Leprosy, and Cancer, Tohoku University, Sendai, Japan.

[4] Mitomycin C: NSC-26980; CAS reg. No. 50-07-7.
2,3,5-Tris(1-aziridinyl)-*p*-benzoquinone: NSC-29215; CAS reg. No. 68-76-8; Trenimon.

[5] Methotrexate: NSC-740; CAS reg. No. 59-05-2; glutamic acid, *N*-[*p*-[[(2,4-diamino-6-pteridinyl)methyl]-methylamino]benzoyl]-.
Cyclophosphamide: NSC-26271; CAS reg. No. 6055-19-2; 2*H*-1,3,2-oxazaphosphorine, 2-[bis(2-chloroethyl)-amino]tetrahydro-, 2-oxide, monohydrate.

Carbazilquinone



$C_{15}H_{19}O_5N_3 = 321.3$

FIGURE 1.—Structural formula of carbazilquinone.

Japan Society for Cancer Therapy in 1970, by Miura et al (2) and Hirota et al (3). However, no clinical report is yet available on the effects of carbazilquinone on solid tumors except for our additional presentation of preliminary results at the same meeting.

We have expanded our clinical experiments on solid tumors with this drug and have obtained interesting findings.

MATERIALS AND METHODS

Methods of Administration

Carbazilquinone given at dose levels of 0.02 or 0.04 mg/kg was dissolved in 20 ml of physiologic saline solution and administered, in most cases, intravenously (iv) in one injection as a single daily dose for as many days as possible. In one case each, intraperitoneal (ip) and intrapleural administration were used in the same way as the iv injection.

Patients Treated

Table 1 summarizes the data on 32 patients who were treated for the first time. Most patients had metastatic or unresectable malignant disease. Twelve patients with gastric cancer and six patients with pulmonary cancer comprised the main part of the study. By the end of May 1971, a total of 41 courses of treatment had been given to 32 patients. Since more than two courses of treatment were conducted in five of these patients (table 2), evaluation of the clinical and side effects was mainly recorded in patients who were being treated for the first time, but evaluation of the effects was not possible in two of these patients due to insufficient dosage of carbazilquinone in one patient and postoperative cancer chemotherapy in the other.

Evaluation Procedures

In order to evaluate the clinical effects more objectively, the criteria of both our Department, Tohoku University (4), and those proposed by the Japan Society for Cancer Therapy were used together. Our Department's criteria are in the form of a numerical grading system based on subjective symptoms and objective findings (table 3). At the time of admission, each positive or objective symptom was counted as three points and was then multiplied by the total number of symptoms present in the patient. This number denotes the index of the clinical symptoms at admission. Successive indexes were obtained whenever symptomatic changes occurred during the subsequent clinical course, by adding the points given to each symptom according to the criteria given in table 3(A). The relative degrees of improvement or aggravation throughout the entire course can thus be presented in a "clinical curve." The average degree of improvement or aggravation is determined by dividing the total points by the total number of symptoms. Clinical responses were evaluated in four grades as shown in table 3(B) according to the average degree of improvement or aggravation.

RESULTS

Clinical Effect

According to our criteria, in 30 patients who were evaluable and received carbazilquinone for the first time the effects of the drug were evaluated as markedly effective in four patients (13.3%), effective in one patient (3.3%), unchanged in 13 patients (43.4%), and ineffective in 12 patients (40.0%) (table 4). The rate of efficacy (markedly effective and effective) was 16.7%.

According to the criteria proposed by the Japan Society for Cancer Therapy, five patients (16.7%) were evaluated as improved, nine patients (30.0%) unchanged, and 16 patients (53.3%) aggravated.

TABLE 1.—Patients who were treated for the first time with carbazilquinone by the end of May 1971

Patient No.	Age, sex	Diagnosis	Previous chemotherapy*	No. of doses/ total dose (mg)	Evaluation of clinical effects	
					Criteria of our Department	Criteria of the Japan Society for Cancer Therapy
1	43, M	Cancer of the stomach† with metastasis to thoracic vertebrae	–	16/31.0	Markedly effective	Improved
2	47, F	Cancer of the ovary† with metastasis to Virchow's node	–	50/49.5	Markedly effective	Improved
3	39, M	Seminoma†	–	15/15.0	Markedly effective	Improved
4	58, M	Cancer of the esophagocardial junction	–	52/52.0	Markedly effective	Unchanged
5	53, F	Hodgkin's disease	–	14/26.0	Effective	Improved
6	51, M	Cancer of the lung	+	95/150.5	Unchanged	Improved
7	61, M	Cancer of the esophagus	–	32/42.0	Unchanged	Unchanged
8	61, M	Cancer of the stomach† with pulmonary metastases	–	27/26.5	Unchanged	Unchanged
9	44, F	Cancer of the lung	–	20/38.5	Unchanged	Unchanged
10	59, F	Cancer of the breast with right pleural effusion	+	28/55.0	Unchanged	Unchanged
11	65, M	Cancer of the lung with metastasis to left cervical nodes	+	7/13.0	Unchanged	Unchanged
12	68, F	Cancer of the lung	+	7/13.0	Unchanged	Unchanged
13	60, F	Cancer of the stomach with ascites and metastases to cervical nodes	–	12/11.5	Unchanged	Unchanged
14	45, M	Cancer of the colon† with liver metastases	+	19/36.0‡	Unchanged	Unchanged
15	71, F	Cancer of the stomach with metastasis to Virchow's node	–	13/24.0	Unchanged	Aggravated
16	37, M	Malignant lymphoma	+	17/17.0	Unchanged	Aggravated
17	67, M	Cancer of the lung	–	20/38.0	Unchanged	Aggravated
18	50, F	Cancer of the stomach†	–	35/47.5	Unchanged	Aggravated
19	69, F	Cancer of the pancreas with liver and pulmonary metastases	–	25/24.5	Ineffective	Aggravated
20	53, M	Cancer of the stomach† with ascites	–	30/30.5	Ineffective	Aggravated
21	54, M	Cancer of the stomach; cancer of the maxilla (surgically treated)	+	50/62.5	Ineffective	Aggravated
22	30, M	Cancer of the stomach with metastasis to mediastinal and right cervical nodes	–	40/39.5	Ineffective	Aggravated
23	59, M	Cancer of the pancreas	–	13/24.0	Ineffective	Aggravated

TABLE 1.—Patients who were treated for the first time with carbazilquinone by the end of May 1971—Continued

Patient No.	Age, sex	Diagnosis	Previous chemotherapy*	No. of doses/ total dose (mg)	Evaluation of clinical effects: Criteria of our Department	Evaluation of clinical effects: Criteria of the Japan Society for Cancer Therapy
24	47, M	Cancer of the bile duct with skin metastases	–	31/60.0	Ineffective	Aggravated
25	38, M	Cancer of the stomach with ascites	+	14/26.0	Ineffective	Aggravated
26	74, M	Cancer of the lung	+	24/48.0	Ineffective	Aggravated
27	45, F	Metastatic liver carcinoma with ascites	–	11/20.0	Ineffective	Aggravated
28	65, F	Cancer of the gall bladder with ascites	–	8/14.0	Ineffective	Aggravated
29	60, M	Myeloblastic crisis of chronic myelocytic leukemia	+	14/14.0‡	Ineffective	Aggravated
30	35, F	Cancer of the stomach and ovary with ascites	–	18/17.0	Ineffective	Aggravated
31	67, F	Sarcoma of the stomach (surgically treated)	–	16/31.0	Patient excluded from clinical evaluation	
32	55, M	Cancer of the stomach with liver metastases	+	5/4.5	Patient excluded from clinical evaluation	

* + = Previous chemotherapy received; – = previous chemotherapy not received.
† Postoperative recurrence.
‡ Carbazilquinone given in combination with prednisolone.

TABLE 2.—Patients who were treated with more than two courses of carbazilquinone by the end of May 1971

Patient No.	Age, sex	Diagnosis	Course No.	No. of doses/ total dose (mg)	Evaluation of clinical effects: Criteria of our Department	Evaluation of clinical effects: Criteria of the Japan Society for Cancer Therapy
2	47, F	Cancer of the ovary with metastasis to Virchow's node	1	50/49.5	Markedly effective	Improved
			2	16/31.0	Unchanged	Unchanged
			3	18/34.0	Effective	Improved
			4*	18/36.0	Markedly effective	Improved
			5*	36/36.0	Effective	Unchanged
1	43, M	Cancer of the stomach with metastasis to the thoracic vertebrae	1	16/31.0	Markedly effective	Improved
			2	7/14.0	Markedly effective	Improved
			3†	12/24.0	Ineffective	Aggravated
5	53, F	Hodgkin's disease	1	14/26.0	Effective	Improved
			2	11/22.0	Effective	Unchanged
15	71, F	Cancer of the stomach with metastasis to Virchow's node	1	13/24.0	Unchanged	Aggravated
			2	21/21.0	Effective	Improved
30	61, M	Cancer of the esophagus	1	32/42.0	Unchanged	Unchanged
			2	18/23.0	Ineffective	Aggravated

* Carbazilquinone given in combination with prednisolone (30 mg/day).
† Carbazilquinone given in combination with 5-FU (250 mg), chromomycin A_3 (NSC-58514) (0.5 mg), and prednisolone (30 mg/day).

TABLE 3.—Criteria for clinical evaluation of the therapeutic results of anticancer agents in our Department (Tohoku University)

A. Evaluation of symptomatic changes

Grade	Definition
0	Disappearance of symptom
1	Marked improvement
2	Slight improvement
3	Unchanged
4	Aggravation
5	Marked aggravation

B. Overall evaiwation of clinical effect based on points assigned to symptoms (see text, Evaluation Procedures, for discussion)

Clinical response	Definition
Ineffective	Degree of aggravation in 4th week is over 3.5
Unchanged	Degree of change in 4th week is between 2.5 and 3.5
Effective	Maximum improvement is between 1.5 and 2.5
Markedly effective	Maximum improvement is below 1.5

TABLE 4.—Clinical effect of carbazilquinone according to our criteria

Tumor type and site	No. of patients treated	Evaluation of clinical effect (No. of patients)				Total No. of patients evaluated	No. of patients excluded from clinical evaluation
		Markedly effective	Effective	Unchanged	Ineffective		
Cancer							
Stomach	12	2		4	5	11	1
Lung	6			5	1	6	
Pancreas	2				2	2	
Bile duct	2				2	2	
Esophagus	1			1		1	
Colon	1			1		1	
Breast	1			1		1	
Ovary	1	1				1	
Malignant lymphoma	2		1	1		2	
Myeloblastic crisis of chronic myelocytic leukemia	1				1	1	
Seminoma	1	1				1	
Others	2				1	1	1
Totals	32	4(13.3%)	1(3.3%)	13(43.4%)	12(40.0%)	30	2
		5(16.7%)					

In individual patients, efficacy or improvement was obtained in two patients with gastric cancer, and one patient each with recurrent ovarian cancer, seminoma, malignant lymphoma, and pulmonary cancer. The efficacy of carbazilquinone was not especially prominent in any specific kind of cancer. Carbazilquinone has rather rapid action and its efficacy may be seen after about 1 or 2 weeks of treatment.

Three patients who had excellent responses are reported later in this paper. In these three patients (Nos. 1, 2, and 3) the first effects after the initial treatment were noted on the 5th day (5 mg total dose), 7th day (14 mg total dose), and 15th day (14.5 mg total dose), respectively.

Table 5 shows the clinical effects of carbazilquinone in 30 first-time treatments, 39 whole series of treatments, and 14 treatments in five patients who were treated more than twice. According to our criteria, the rate of efficacy was 16.7% in patients who were treated for the first time, and 28.2% in the whole series of treatments. The rate was as high as 64.3% in 14 treatments of five patients who were treated more than twice. Similar results were obtained according to the criteria of the Japan Society for Cancer Therapy. These results indicate that administration could not be continued due to leukopenia, and re-administration of the drug after the white blood cell count (wbc) returned to normal gave the same results again in some cases.

Frequency of Administration and Total Dosage

Table 6 summarizes the frequency of administration of carbazilquinone and the total dosage. Among 31 patients, the minimum dose was 11.5 mg and the maximum dose was 150.5 mg, with an average dose level of 35.4 mg. The average dose levels in patients who responded and patients with leukopenia (< 3000 cells/mm^3) were 34.7 and 43.3 mg, respectively. The dose level was slightly higher in patients

TABLE 5.—Comparison of the clinical effects of carbazilquinone

Evaluation rating	No. of patients responding/ total No. treated for first time	No. of patients responding/ total No. treated	No. of patients responding/ total No. treated more than twice
Markedly effective + effective*	5/30 (16.7%)	11/39 (28.2%)	9/14 (64.3%)
Improved†	5/30 (16.7%)	9/39 (23.1%)	7/14 (50.0%)

*Criteria of our Department (Tohoku University).
†Criteria of Japan Society for Cancer Therapy.

TABLE 6.—Frequency of administration and total dose

	Frequency of administration		Total dose (mg)	
	Range (days)	Average (days)	Range (days)	Average (days)
Total patients treated for the first time (31 patients)	7–95	24.9	11.5–150.5	35.4
Patients with markedly effective or effective treatment (5 patients)	14–52	29.5	15.0–52.0	34.7
Patients rated improved (5 patients)	14–95	38.0	15.0–150.5	54.4
Patients with leukopenia (18 patients)	12–95	28.7	11.5–150.5	43.3

with leukopenia and in improved patients, probably because these groups included one patient with pulmonary cancer in whom it was possible to give an exceptionally high dose, 150.5 mg. When this patient was excluded, no marked difference was found in the total dose among the whole series, the patients who responded, and the patients with leukopenia. This would indicate that due to leukopenia, discontinuation of the drug was inevitable in many cases regardless of positive or negative effects. In patients who were treated for a long period of time, a response was not always obtained.

Side Effects and Reasons for Drug Discontinuation

Side effects were studied in 31 patients, including one patient who was treated postoperatively. As shown in table 7, leukopenia (< 3000 cells/mm³) represented the main side effect (18 of 31 patients; 58.1%). Thrombocytopenia (<50,000 platelets/mm³) developed in seven patients (22.6%). Anorexia developed in five patients (16.1%). Anemia developed in two patients, and one case each of hemorrhagic tendency, nausea and vomiting, and general malaise were also observed. The most frequent reason for discontinuing therapy was leukopenia which developed in 18 patients (58.1%) (table 8).

In order to see the fluctuations in toxic effects to the liver and kidney in response to carbazilquinone administration, icterus index, serum glutamic oxaloacetic transaminase, serum glutamic pyruvic transaminase, thymol turbidity test, zinc sulfate turbidity test, alkaline phosphatase, total cholesterol, nonprotein nitrogen, blood urea nitrogen, phenolsulfonphthalein test, and electrolytes (Na, K, Cl, and Ca) of serum were compared before and after the administration of this drug. No definite influence was noted besides the fluctuations due to the change of the disease itself.

TABLE 7.—Toxic side effects of carbazilquinone

	No. of patients with toxic effect/ total No. treated	Percent
Leukopenia (<3000 cells/mm³)	18/31	58.1
Thrombocytopenia (<50,000 platelets/mm³)	7/31	22.6
Anemia	2/31	6.5
Hemorrhagic tendency	1/31	3.2
Anorexia	5/31	16.1
Nausea and vomiting	1/31	3.2
General malaise	1/31	3.2

TABLE 8.—Reasons for discontinuation of therapy

	No. of patients/ total No.	Percent
Leukopenia (<3000 cells/mm³)	18/31	58.1
Thrombocytopenia (<50,000 platelets/mm³)	1/31	3.2
No effect	6/31	19.4
Death	4/31	12.9
Discharge	2/31	6.5

Since hematopoietic toxic effects are inevitable with the use of alkylating agents, the influence on the peripheral blood and the bone marrow was also studied.

Leukopenia occurred in over half of the patients and the decrease in leukocyte counts continued even after the discontinuation of the medication. The recovery from leukopenia was rather slow and it usually took several weeks for the patient to recover. When the group that was treated with 0.02 mg/kg/day and the group that was treated with 0.04 mg/kg/day were compared, the slope of the decrease curve was not definitely different between the two groups, but the recovery from the leukopenia was faster in the group treated with 0.02 mg/kg/day. The platelet count also fluctuated in a tendency similar to that of the wbc. However, thrombocytopenia did not appear to be as pronounced as leukopenia.

Figure 2 shows the changes of the nucleated cell count in the bone marrow aspirate before and after the administration of the drug. In most cases, nucleated cells in the bone marrow were fewer at the time that therapy was discontinued because of leukopenia, which indicates a marked inhibitory effect of carbazilquinone on the bone marrow.

Comparison of Clinical and Side Effects After a Single Administration

In the group given a single dose of 0.02 mg/

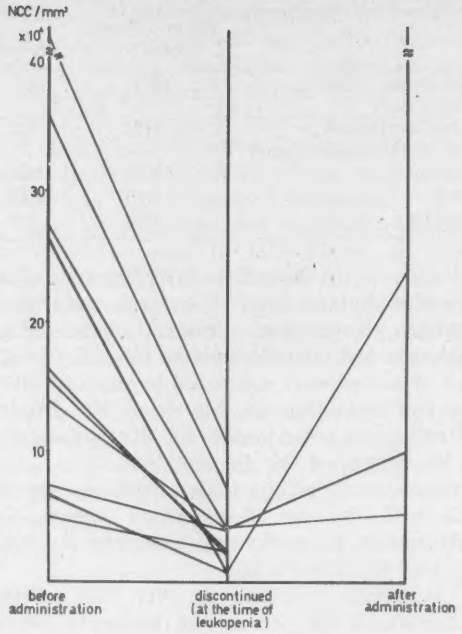


FIGURE 2.—Changes in nucleated cell count in the bone marrow before and after administration.

kg/day and in the group given 0.04 mg/kg/day, the clinical effects and the rate of occurrence of leukopenia and thrombocytopenia were compared. As shown in table 9, ten patients were treated with carbazilquinone for the first time at dose levels of 0.02 mg/kg/day, and 16 patients were treated with 0.04 mg/kg/day. The average total doses were 28.3 and 31.1 mg, respectively, with scarcely any difference between the two groups. However, the rate of occurrence of leukopenia was 50.0% in the former group and 62.5% in the latter group; the thrombocytopenia rate was 10.0% in the former group and 35.7% in the latter group. The rate of occurrence of both leukopenia and thrombocytopenia thus appears to be higher in the group that was treated with 0.04 mg/kg/day. The rate of efficacy, however, was 30.0% in the group treated with 0.02 mg/kg/day but 13.3% in the group treated with 0.04 mg/kg/day. The rate of improvement was 20.0% in the former group and 13.3% in the latter group, the rate of improvement being higher in the former than in the latter.

A similar tendency was noted between the total number of treatments of the two groups. Consequently, it seems likely that the administration of 0.02 mg/kg/day is better from the viewpoint of clinical and side effects.

CASE REPORTS

Three patients with excellent responses are presented.

Case No. 1—A 43-year-old man (patient No. 1) who suffered from postoperative recurrence of gastric cancer with metastases to the fourth thoracic vertebra and to Virchow's node (0.8 × 0.7 cm) was treated with carbazilquinone at a dose level of 0.04 mg/kg/day. The chief symptoms were back pain and motor and sensory disturbance below the level of the umbilicus. Rapid improvement took place within 1 week; these symptoms and the swollen metastatic Virchow's node disappeared when

TABLE 9.—Comparison of clinical and side effects after single administration

	Dose	Average No. of administrations	Average total dose (mg)	Incidence of leukopenia (%)	Incidence of thrombocytopenia (%)	Percent of patients with clinical effects	
						Efficacy	Improved
Patients treated for the first time (10 patients)	0.02 mg/kg/day	28.6	28.3	50.0	10.0	30.0	20.0
Total patients treated (12 patients)		28.6	28.3	58.3	18.2	41.7	25.0
Patients treated for the first time (16 patients)	0.04 mg/kg/day	16.3	31.1	62.5	35.7	13.3	13.3
Total patients treated (22 patients)		15.6	29.9	68.2	35.0	27.3	22.7

leukopenia necessitated interruption of the treatment on the 16th day. The effect of the drug was evaluated as markedly effective. From the 10th day of discontinuation, however, pain reappeared along with symptoms of spinal cord transection below the breast level, accompanied by bladder and rectal disturbance. Re-administration of carbazilquinone at the same dose level seven times resulted in the almost complete disappearance of these symptoms, which again indicated the excellent effect of carbazilquinone. Four months later the patient died. Postmortem examination showed epidermoid carcinoma of the residual stomach with metastases to the thoracic and lumbar vertebrae.

Case No. 2—A 47-year-old housewife (patient No. 2) with postoperative recurrence of ovarian cancer (papillary adenocarcinoma) showed a high sensitivity to carbazilquinone. Including the two times that prednisolone[6] was given concomitantly, carbazilquinone was given five times in this case. At admission, metastasis to Virchow's node was palpable (0.7 × 0.7 cm). Tenderness and tumorous resistance were noted on the left side of the abdomen. Carbazilquinone was given at a dose level of 0.02 mg/kg/day. From around the 15th day, the swollen lymph node tended to become smaller. The swelling of Virchow's node and tumorous resistance in the abdomen disappeared by the time the treatment was discontinued because of the development of leukopenia on the 50th day. Six weeks later, carbazilquinone was given again for the purpose of consolidation at a dose level of 0.04 mg/kg/day for 16 days (fig 3). The clinical results of the next three courses of treatment with

[6] Prednisolone: NSC-9900; CAS reg. No. 50-24-8; pregna-1,4-diene-3,20-dione, 11β,17,21-trihydroxy-.

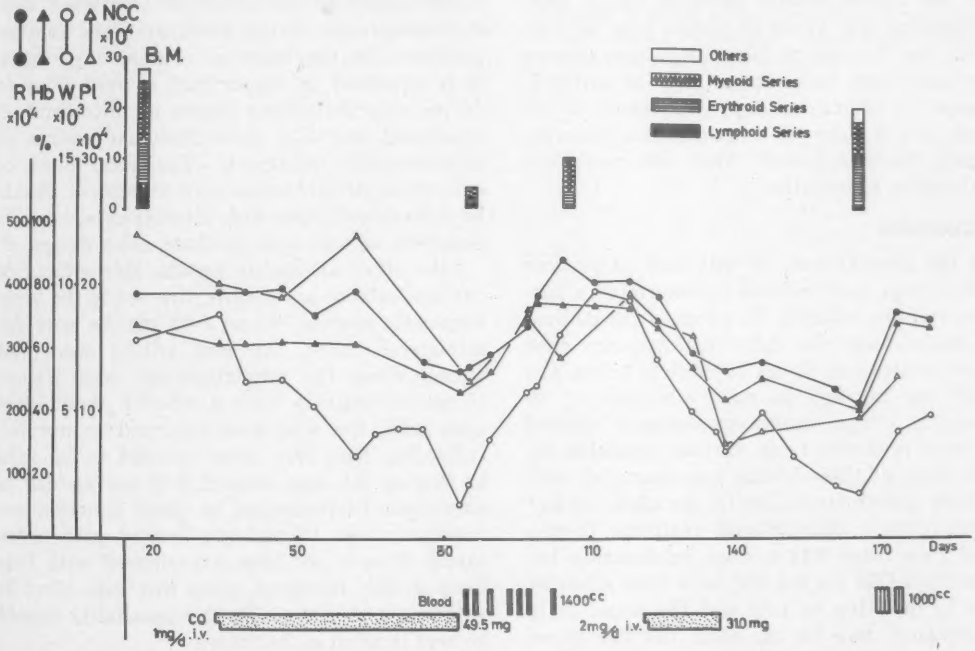


FIGURE 3.—Case No. 2: fluctuations in peripheral and bone marrow pictures in response to carbazilquinone.

carbazilquinone were evaluated as effective, markedly effective, and effective, respectively (table 2). Thirteen months after admission the patient died. Postmortem examination revealed a large amount of bloody ascites and a large mass of greater omentum with cancerous infiltration as the main macroscopic findings.

Case No. 3—A 39-year-old man (patient No. 3) was admitted with abdominal pain and lumbago from recurrent seminoma. A 9 × 12-cm tumor at the upper abdomen; a 12 ×12-cm tumor at the lower abdomen, marked venous patterns over the upper abdomen, and edema of the legs were the main physiologic findings at admission. The patient was treated with carbazilquinone at a dose level of 0.02 mg/kg/day. On the 5th day, abdominal pain improved and tumors became smaller on the 8th day. After 15 administrations, leukopenia necessitated interruption of the treatment. At that time abdominal pain and lumbago disappeared and the tumors became unpalpable. Thirty days later, abdominal pain appeared and 40 days later the tumor became palpable again. Carbazilquinone was given at a dose level of 0.02 mg/kg/day for 6 days. Since no pain or tumors were persistent thereafter, 1 mg of carbazilquinone (0.02 mg/kg/day) was used at intervals of 1 or 2 days as a maintenance therapy, keeping the wbc around 3000 cells/mm^3; the case is under observation.

DISCUSSION

In the clinical trial of this new anticancer agent, it has been difficult to determine a satisfactory dose schedule. To prepare the method for establishing the daily maintenance dose of new anticancer drugs in clinical trials, the LD50s (in mg/kg) in mice and rats of 29 anticancer drugs under conventional clinical use were reviewed from various available reports. Each of these LD50s was compared with the daily maintenance dose for an adult (50 kg) treated with a conventional regimen. It was found that there was a close relationship between the LD50 (in mg/kg) of a drug given by iv or ip injection to rats and the usual daily maintenance dose for an adult (50 kg) given by iv or intracavitary injection. Therefore, when a new anticancer drug was employed clinically for the first time in our Department, the LD50 (in mg/kg) in rats, or a dose close to this value, was considered as the daily maintenance dose for an adult (50 kg). The clinical trial was started at a dose level of about one seventh to one third of the LD50 (in mg/kg) in rats and was followed by a gradual increase with a careful observation of side effects until the proposed daily dose was reached 1 week later. Our study, like the one just described, was reported elsewhere (6).

The LD50 (ip) of carbazilquinone in rats is 4.44 mg/kg. Suspecting the side effects of carbazilquinone on the hematopoietic system, 0.04 mg/kg (2 mg/50 kg) was established as the initial daily maintenance dose. Marked bone marrow depression later necessitated a decrease of the maintenance dose to 0.02 mg/kg (1 mg/50 kg). Although 0.04 mg/kg/day (2 mg/50 kg) was selected as the daily dose in the beginning, 0.02 mg/kg/day (1 mg/50 kg) appears to be better with regard to the clinical and side effects, as seen in the results of the present study.

The extent of one course of treatment with carbazilquinone in the adult presents another problem. On the basis of clinical experience, it is admitted in Japan that a total dose of 40 mg constitutes one course of mitomycin C treatment and 5 g constitutes one course of 5-fluorouracil treatment.[7] The total dose of one course of carbazilquinone treatment should be determined after wide clinical experience is obtained, as was done in these older drugs.

Like other alkylating agents, side effects of carbazilquinone are mainly directed to the hematopoietic system. When 0.02 mg/kg was administered daily, the side effects were not lethal. When the administration was discontinued in patients with a wbc of about 3000 cells/mm^3, the wbc soon returned to normal, indicating that this drug appears to be safe as long as the dose level of 0.02 mg/kg/day is employed. Disturbances in renal function occasionally seen in patients treated with mitomycin C have not been experienced with this drug so far. However, since this side effect is rather late in appearing, this possibility should be kept in mind in the future.

As described earlier, carbazilquinone is of

[7] 5-Fluorouracil: NSC-19893; CAS reg. No. 51-21-8.

value clinically, with the possibility of widespread clinical use in the future. Since this drug has a chemical structure quite similar to that of mitomycin C, which has such components with anticancer activity as the aziridine, the urethane, and the quinone groups, comparison with the clinical responses to mitomycin C will become a problem. Such a comparison is an important part of the development of carbazilquinone, but it would be premature at present, because clinical information comparing carbazilquinone to mitomycin C is still extremely poor.

The combined use of many drugs, local irrigation, and the development and improvement of methods of administration all contribute to more successful chemotherapy, but the development of a more effective and safer anticancer agent is of fundamental importance. Nevertheless, no anticancer agent definitely superior to the conventionally used drugs has yet appeared despite many efforts. Carbazilquinone, which was developed as the result of synthesizing a combination of structural groups with anticancer activity, should be highly evaluated. In the future, such combination of active anticancer structural groups through various synthetic procedures might be a new mode of producing a new anticancer agent with a greater clinical effect and fewer side effects.

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Cis-diamminedichloroplatinum (NSC-119875): A Phase I Study [1,2,3,4]

Donald J. Higby, H. James Wallace, Jr., and James F. Holland [5]

SUMMARY

Forty-five patients with intractable malignant disease were treated with *cis*-diamminedichloroplatinum. Nausea and vomiting occurred at all dose levels of the drug. At the higher dose levels, sporadic white blood cell count and platelet count depressions were seen. Tinnitus and high-frequency hearing loss occurred in four patients. At dose levels of 24 mg/m² given daily for 5 days or 100 mg/m² given daily for 1 day, creatinine elevations consistently occurred, and in a few cases the elevations were prolonged or irreversible. Postmortem examination revealed that renal tubular necrosis had occurred in two patients within 1 month of drug administration, and tubular swelling was noted in a third patient. Tumor regressions were seen in one patient with breast cancer, one with transitional cell carcinoma of the bladder, one with anaplastic (small cell) thyroid carcinoma, and nine of 11 patients with testicular tumors. The schedule chosen for phase II studies was 20 mg/m² of *cis*-diamminedichloroplatinum given daily for 5 days every 4 weeks, if the renal, hematologic, and auditory functions permitted.

[Cancer Chemother Rep 57:459–463, 1973]

Cis-diamminedichloroplatinum is one of a group of coordination compounds of platinum identified by Rosenberg and Van Camp (1), which strongly inhibits bacterial replication. The structure is shown in figure 1.

Howle and Gale (2) showed that *cis*-diamminedichloroplatinum suppressed thymidine uptake in Ehrlich ascites tumor cells for up to 96 hours, while only transiently affecting uridine and *l*-leucine uptake. Such an effect could be attributed to a persistent and selective binding to DNA causing irreversible cross linkage. In short, the drug appears to act in a manner similar to an alkylating agent.

Toxicity studies were performed by Heyman et al (3). The LD50 for a single intravenous (iv) dose in Swiss mice was 13.38 mg/kg with death occurring between 2 and 8 days after the start of treatment. The minimum lethal dose in dogs was 2.5 mg/kg by single iv injection and was 0.75 mg/kg/day when the drug was administered for 5 days. The minimum toxic dose for dogs was 22.5 mg/m² given as a single dose. The minimum lethal dose in monkeys was 1.25 mg/kg given daily for 5 days, and the minimum toxic dose was 4.2 mg/m² given daily for 5 days.

In dogs and monkeys, the major manifestations of toxicity were hemorrhagic enterocolitis, severe bone marrow hypocellularity, lymphoid atrophy, and renal tubular necrosis.

[1] Received June 13, 1973; accepted July 19, 1973.

[2] Presented in part at the annual meeting of the American Society of Clinical Oncology, Apr 10, 1973.

[3] Supported by Public Health Service grant CA-05834 from the National Cancer Institute (NCI), National Institutes of Health (NIH), Department of Health, Education, and Welfare.

[4] *Cis*-diamminedichloroplatinum: CAS reg. No. 1853-33-9. Supplied by Cancer Therapy Evaluation, Division of Cancer Treatment, NCI.

[5] Department of Medicine A, Roswell Park Memorial Institute, Buffalo, NY.

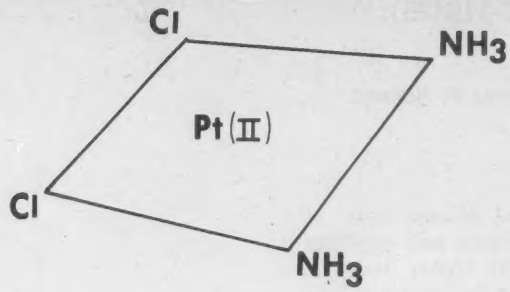

FIGURE 1.—Structure of *cis*-diamminedichloroplatinum.

Testicular atrophy was seen in one monkey. Evidence of antitumor activity was demonstrated by regression of DMBA-induced mammary tumors in female rats (4), large established solid Sarcoma 180 tumors (5,6), Dunning ascitic leukemia, and Walker 256 carcinosarcoma (7). The effects on L1210 ascitic leukemia have been explored by several investigators (5,8–10), and a consistent increase in the life-span and occasional cures have been observed.

A phase I study was carried out at Roswell Park Memorial Institute in which 45 patients, with malignant disease that was unresponsive or refractory to conventional therapy, were treated with *cis*-diamminedichloroplatinum in escalating doses. A clinically tolerable dose schedule was developed for further exploration of the antitumor spectrum and toxicity of this new agent.

PLAN OF STUDY

Cis-diamminedichloroplatinum was supplied in ampules containing 10 mg of the powder, which was reconstituted with 10.0 ml of sterile water for injection, and was given by immediate, rapid iv infusion over a period of 3–5 minutes.

Patients with advanced cancer who had either exhausted other forms of therapy or who had tumors known to be resistant to other forms of therapy, were selected for initial clinical trials. Requirements for entry included complete blood cell counts, normal creatinine clearances, and an estimated survival of 2 months. All patients were informed of the nature of the study and possible side effects.

Two dose schedules were explored. Schedule I involved giving a single large dose of *cis*-diamminedichloroplatinum and repeating it at intervals determined by the subsidence of toxicity. The initial dose was 4 mg/m², which was one sixth the minimal toxic dose in dogs (3). This dose was given to two patients who were observed for 2 weeks. The dose was then escalated and administered to two more patients until the toxic effects were consistent and reversible.

Schedule II involved giving the drug daily for 5 days and repeating this as frequently as practical after evidence of toxicity had subsided. The initial dose employed in this schedule was 1.5 mg/m² of body surface area given daily for 5 days. This dose represented one third of the minimum toxic dose in monkeys (3). As in schedule I, the dose was administered to two patients, and these patients were observed for 2 weeks. This dose was then escalated by a modified Fibonacci search and two more patients were treated and observed. This schedule of therapy was also continued until consistent reversible toxicity was observed.

RESULTS

Tables 1 and 2 indicate, respectively, the toxicity associated with increasing doses of *cis*-diamminedichloroplatinum in schedules I and II.

Nausea and vomiting occurred with virtually every course of the drug, beginning 1 or 2 hours after injection and persisting for a variable period of time. Renal dysfunction (elevations of blood urea nitrogen [BUN] and serum creatinine values, and depression in creatinine clearance) appeared to be dose-related and were first noted between 7 and 14 days after the initiation of therapy. In this study, white blood cell count (wbc) and platelet count depressions occurred sporadically and were not dose-limiting. Tinnitus occurred in two patients and a high-frequency hearing loss demonstrated by audiography could be determined in these and two other patients, indicating mild eighth-nerve damage. These symptoms resolved completely in each patient. At the dose levels of 24 mg/m²/day for 5 days and 100 mg/m² in a single dose, prolonged, probably irreversible serum creatinine eleva-

TABLE 1.—Schedule I: single-dose treatment

No. of patients entered	Dose level (mg/m^2)	No. of courses	Toxic effects
1	4.0	1	Nausea
1	8.0	4	None
3	50*	9	Nausea and vomiting in all patients. BUN from <15 to >35 mg/100 ml in two of three patients. Serum creatinine elevation to 4.4, 2.8, 2.1 mg/100 ml in three patients (returned to normal). Wbc from 5500 to 1200 cells/mm^3, and from 5800 to 2600 cells/mm^3 in two of three patients (reversible).
3	75	4	Nausea and vomiting in all. Serum creatinine elevations in two of three patients (2.9 and 3.2 mg/100 ml). Wbc from 3800 to 2800 cells/mm^3 in one patient; 4300 to 2700 cells/mm^3 in another. All values returned to normal.
9	100	13	Nausea and vomiting in all. Serum creatinine elevations (2.0–3.0 mg/100 ml); creatinine values did not return to normal in five of nine patients. Wbc depressions to 2000–3000 cells/mm^3 in two patients. Severe persistent tinnitus in one. High-frequency hearing loss in two. Protracted anorexia in one.

*At this level other investigators had reported tolerance to single doses as high as 100 mg/m^2 (11), consequently, the next dose level given in our trial was 50 mg/m^2.

TABLE 2.—Schedule II: 5-day course

No. of patients entered	Dose level (mg/m^2)	No. of courses	Toxic effects
2	1.5	2	Nausea
3	3.0	3	Nausea
2	5.0	4	Nausea; in one patient wbc on Day 16 was 2100 cells/mm^3.
3	7.5	5	Nausea; in one patient platelet count dropped from 240,000 to 90,000 cells/mm^3 by Day 26. BUN rose to 34 mg/100 ml, uric acid to 14.4 mg/100 ml; concomitant dehydration.
2	10.0	6	Nausea and vomiting
2	12.5	3	Nausea; in one patient platelets dropped from 250,000 to 75,000 cells/mm^3 by Day 29. Serum amylase rose to 380 units/100 ml.
2	18	5	Nausea; in one patient creatinine clearance dropped from 76 to 55 ml/min by Day 42. In the other patient there was no change.
9	20	14	Nausea and vomiting in eight. No auditory toxicity. After one course, renal toxicity developed in three patients (one had gentamicin therapy at the same time). After more than one course, renal toxicity developed in four other patients. Two had prolonged elevations of serum creatinine (23 mos).
3	24	3	Nausea and vomiting; in two of three patients, serum creatinine elevations (2.0 and 1.7 mg/100 ml). Severe tinnitus with 20% high-frequency hearing loss in one patient.

tions took place in two of three patients in the former group and five of nine patients in the latter group.

In the group that received 20 mg/m² daily for 5 days, only three of the nine patients showed evidence of renal toxicity after the first course of therapy, and in each case this toxicity was reversible. In those who received more than one course, renal toxicity occurred in seven of nine patients, and two showed prolonged, presumably irreversible toxicity.

Renal tubular necrosis was demonstrated histologically in two patients who died during the study. One of these patients had received 100 mg/m² in a single dose, and one had received 20 mg/m² daily for 5 days. Both patients had received brief courses of gentamicin shortly before death. A third patient, who had also received *cis*-diamminedichloroplatinum at a dose level of 20 mg/m² daily for 5 days, underwent a needle biopsy of the kidney during a time when the serum creatinine was elevated, and this revealed tubular swelling under light microscopy.

ANTITUMOR EFFECTS

During this study and subsequent explorations, complete regressions were noted in one patient with transitional cell carcinoma of the bladder and in one patient with small cell thyroid carcinoma. Improvement was seen in one patient with breast cancer.

Eleven patients with testicular tumors were treated, and three of 11 patients (one with seminoma, one with embryonal cell carcinoma, and one with choriocarcinoma) showed complete regression of tumor. Three other patients (two with embryonal cell carcinoma and one with choriocarcinoma) showed partial regression. Of the remaining five patients, three showed objective improvement with a less than 50% regression of tumor (10). Figure 2 illustrates the antitumor effect of *cis*-diamminedichloroplatinum in a patient with embryonal cell carcinoma.

CONCLUSION

Cis-diamminedichloroplatinum can be administered with reasonable safety provided that

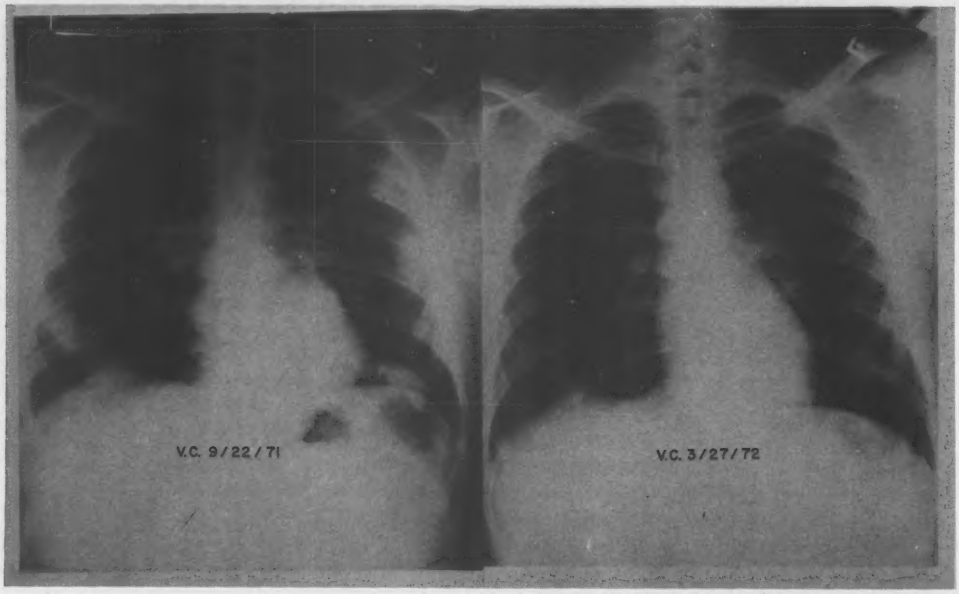

FIGURE 2.—Antitumor effect in a patient with embryonal cell carcinoma of the testes.

kidney function is frequently and carefully assessed. The side effects, both immediate and long term, are tolerable at dose levels in which an antitumor effect is seen. These toxic side effects are chiefly renal injury, transient eighth-nerve toxicity, nausea and vomiting, and occasional hematologic depression. Since hematologic toxicity is mild and sporadic at dose levels which are toxic to the kidneys, the drug may be useful in combinations with agents which are more myelosuppressive.

A significant degree of antitumor effect has been demonstrated by *cis*-diamminedichloroplatinum, especially among testicular tumors. Further explorations of the clinical usefulness of this drug are being undertaken at Roswell Park Memorial Institute. Emphasis will be on reducing the renal toxicity by modifying the dose schedule, looking for synergism in drug combinations, and enhancing the excretion of the heavy metal.

The suggested dose schedule for further phase II studies is 20 mg/m² given iv daily for 5 days once per month until renal toxicity supervenes.

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Clinical Evaluation of Toxic Effects of *Cis*-diamminedichloroplatinum (NSC-119875)—Phase I Clinical Study [1,2,3,4]

R. W. Talley, R. M. O'Bryan, Jordan U. Gutterman, R. W. Brownlee, and K. B. McCredie [5,6,7]

SUMMARY

Sixty-five patients were entered into a phase I clinical study of *cis*-diamminedichloroplatinum to determine a safe dose regimen that would produce mild to moderate toxic effects in a majority of patients. Since preclinical tests determined that the kidneys, the gastrointestinal tract, and the hematopoietic system were the most vulnerable, these systems were observed most carefully. Fifty-seven patients survived for 4 weeks after initiation of therapy and were evaluable for toxic effects. Three patients died of advanced cancer in less than 4 weeks, and in five patients, one complete course of therapy was not completed. Patients with a wide variety of neoplasms were randomly treated with either of two dose regimens of the drug: (*a*)treatment for 5 days every 28 days or (*b*) treatment twice weekly for 3 weeks. The initial dose was 1.0 mg/m^2 which was escalated in subsequent patients to a maximum dose of 20 mg/m^2. Renal toxicity increased in incidence with the higher doses, as shown by increased blood urea nitrogen or serum creatinine levels or both. Extrarenal factors did not appear to be apparent in the elevation of these parameters. Hematologic toxicity was definitely dose-related and may have been cumulative. Gastrointestinal toxicity, primarily nausea or vomiting or both, increased with larger doses but results were not as clear as those observed with renal and hematopoietic toxicity. The dose schedule of 15 mg/m^2 for 5 days each month was acceptable to most patients. Three of five patients with lymphomas had objective remissions of short duration ranging from 1 to 3 months, one patient with a neuroblastoma had a 2-month regression, and one patient with a malignant thymoma had an objective remission which lasted 13 months. Further exploration in more patients and possibly the evaluation of combinations of *cis*-diamminedichloroplatinum with other drugs are recommended.

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[2] Presented in part at the Second International Symposium on Platinum Coordination Complexes in Cancer Chemotherapy, Apr 1973, University of Oxford, England.

[3] Supported in part by Public Health Service grants CA-04915, CA-03392, CA-10376, and CA-03754 from the National Cancer Institute (NCI), National Institutes of Health, Department of Health, Education, and Welfare.

[4] *Cis*-diamminedichloroplatinum was supplied by Cancer Therapy Evaluation, Division of Cancer Treatment, NCI. Chemical nomenclature, NSC numbers, and CAS registry numbers for all compounds mentioned in this paper are listed in the List of Compounds at the end of the text.

The inhibition of cell division when *Escherichia coli* cells are exposed to electrolysis products from a platinum electrode was first reported in 1965 by Rosenberg, Van Camp, and Krigas (1). This same group subsequently studied several ammonium platinum salts in transplanted Sarcoma 180 (2) and found that some of these platinum salts were capable of inhibiting tumor growth significantly in this animal model system. Later, other investigators confirmed the activity of platinum salts in a wide variety of transplanted rodent tumor models. The most consistently potent analog, *cis*-diamminedichloroplatinum, was selected for extensive toxicologic studies in rodents, dogs, and monkeys to determine preclinical toxic effects. The highest nontoxic dose for dogs was 3.87 mg/m²/day for 5 days. Monkeys appeared to be somewhat more sensitive, with the highest nontoxic dose being 1.94 mg/m²/day for 5 days. However, the lethal dose for both dogs and monkeys was similar: 31.3 mg/m² for dogs and 33.6 mg/m² for monkeys. At the higher doses, the major sites involved in animal toxicologic studies were kidney, gastrointestinal tract, lymphatics, and bone marrow. As a result of these studies and reports, a phase I clinical trial was started under the auspices of the Southwest Oncology Group (SOG) in June 1971.

METHODS

The drug was supplied in vials containing 10 mg of *cis*-diamminedichloroplatinum, 90 mg of sodium chloride, and 100 mg of mannitol. It was reconstituted with 10 ml of sterile water and was administered directly intravenously (iv) at the appropriate dose level. Patients selected for this trial were required to have a white blood cell count (wbc) over 5000 cells/mm³, a platelet count over 125,000 platelets/mm³, and a blood urea nitrogen (BUN) level less than 22 mg/100 ml. Other requirements were: no prior chemotherapy within 4 weeks of this trial, a predicted survival time of at least 6 weeks, and a Karnofsky performance status of 50% or better. All patients were required to sign an informed consent acknowledging that they were receiving an investigational drug.

The following laboratory studies were required for all patients prior to initiation of therapy: complete blood cell count, platelet count, reticulocyte count, and a bone marrow aspiration; urinalysis; BUN; and serum creatinine and urea clearance. In addition, liver function studies such as serum glutamic oxaloacetic transaminase, lactic dehydrogenase, alkaline phosphatase, bilirubin, and sulfobromophthalein were required.

Patients were randomly assigned to either of two dose regimens: (*a*) twice weekly for 3 weeks or (*b*) daily for 5 days and repeated 4 weeks after the initial dose. The dose-escalation schedule was initially established with patients on the twice-weekly schedule receiving either 1.0, 1.5, 3.0, 5.0, 6.0, 7.5, 8.0, 10.0, 12.0, or 15.0 mg/m². On the 5-day-a-week schedule, the dose escalation was 1.0, 3.5, 4.0, 5.0, 6.0, 7.5, 10.0, 12.0, 15.0, 17.5, and 20.0 mg/m². On either dose regimen, those patients who received less than 10.0 mg/m² and who had neither significant toxic effects nor response were escalated to the next higher dose level. The courses were repeated at 28-day intervals. Randomization was carried out in the Statistical Office of the SOG. During the course of chemotherapy, patients were observed closely for any signs of gastrointestinal disturbance (such as nausea, vomiting, or diarrhea) or other manifestations of clinical tox-

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[*] This study was carried out under the auspices of the Southwest Oncology Group in June 1971. Institutions participating in this study were: Henry Ford Hospital, Detroit, Mich (Drs. Talley, O'Bryan, and Brownlee); M. D. Anderson Hospital, Houston, Tex (Drs. Gutterman and McCredie); Lackland Air Force Base, Wilford Hall USAF Medical Center, Tex; and Baylor University, College of Medicine, Houston, Tex.

[†] The authors wish to thank Mrs. Anne C. Ulbrich for her assistance in the editing and preparation of this paper.

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icity, and for changes in the size of tumor masses. Some of the previously mentioned laboratory studies were repeated twice weekly for a period of 2 weeks after the completion of the course. In some patients, a bone marrow aspiration was repeated 24 hours after they had received the last dose of the drug. Observations were recorded on standard SOG flow sheets and were submitted to the study coordinator for analysis. A large majority of the patients in the study were from Henry Ford Hospital and M.D. Anderson Hospital.

The major objective of this phase I clinical trial was to determine a safe dose regimen which would produce mild to moderate toxic effects in a majority of the patients. Because preclinical tests had determined that the kidneys, gastrointestinal tract, and hematopoietic system were the most likely targets for insult, these systems were observed most carefully by all investigators. The following definitions of toxicity were used:

Hematopoietic

(*a*) Mild: depression of wbc to 2500–3500 cells/mm^3 or platelet count to 75,000–100,000 platelets/mm^3 or both.

(*b*) Moderate: depression of wbc to 1500–2500 cells/mm^3 or platelet count to 50,000–75,000 platelets/mm^3 or both.

(*c*) Severe: depression of wbc to less than 1500 cells/mm^3 and platelet count to less than 50,000 platelets/mm^3.

Renal

(*a*) Mild: increase of BUN to 25–35 mg/100 ml or serum creatinine to 1.5–2.0 mg/100 ml or both.

(*b*) Moderate: increase of BUN to 36–50 mg/100 ml or serum creatinine to 2.1–2.5 mg/100 ml or both.

(*c*) Severe: increase of BUN over 50% or serum creatinine over 2.5 mg/100 ml or both.

Gastrointestinal

(*a*) Mild: of such a nature that concomitant medication for control of symptoms was not necessary.

(*b*) Moderate: required varying amounts of antiemetic drugs to control nausea or vomiting or both.

(*c*) Severe: symptoms not controlled by vigorous supportive medication.

Even though objective tumor responses were not the primary objectives of the study, decreases in the sum of the product of the diameters of all measurable tumor masses by 50% or greater were classified as objective remissions. Decreases of 25%–50% were recorded, but were not considered objective remissions. The presence of measurable disease was not a requirement for entry of a patient into the study.

RESULTS

Toxicity

Sixty-five patients were entered into the study; 57 patients were eligible for evaluation of toxicity, ie, they had survived for 4 weeks after the start of therapy. Three patients died in less than 4 weeks, four patients did not complete at least one full course, and in one patient the data was insufficient for evaluation. Since one of the purposes of the project was to see if there was any possible schedule-dependency in relation to human toxicity, the two different dose schedules were selected. As can be seen in table 1, the hematologic toxicity was definitely dose-related and may be cumulative. The number of courses given at the lower dose levels was combined because of the relative infrequency of any significant toxicity. However, of the five patients treated with 17.5 mg/m^2 on the 5-day schedule, moderate to severe toxicity was observed in three patients. The dose of 15 mg/m^2, either on the 5-day schedule or the twice-weekly schedule, appeared to be fairly well-tolerated with only mild to moderate toxicity occurring in 15 courses in 12 patients.

Renal toxicity, as shown by increasing BUN or creatinine levels (table 2), also increased in incidence with the higher dose regimens. Gastrointestinal toxicity, primarily nausea or vomiting or both, increased in incidence with larger doses, but the results were not as clear as those observed with renal and hematopoietic toxicity.

TABLE 1.—Evaluation of hematologic toxicity of *cis*-diamminedichloroplatinum on the 5-day schedule

Dose (mg/m^2)	No. of patients	No. of courses	Wbc (cells × $10^3/mm^3$)		Platelets (cells × $10^4/mm^3$)		Fall in hemoglobin level
			2.5–3.5	<2.5	5.0–10	<5	(>2 g)
Low-dose escalation							
1.0–5.1	8	11	1	0	1	0	0
6.0–12.0	7	11	1	2*	0	2*	1
High-dose escalation							
15.0	10	15	2	0	4	0	2
17.5	5	5	0	3	2	1	2
20 0	1	1	0	1	0	1	1

*On 10.0-mg/m^2 schedule.

TABLE 2.—Evaluation of renal and gastrointestinal toxicity of *cis*-diamminedichloroplatinum on the 5-day schedule

Dose (mg/m^2)	No. of patients	No. of courses	Renal toxicity: BUN level (mg/100 ml)			Renal toxicity: Creatinine level (mg/100 ml)			Gastrointestinal toxicity		
			25–35	35–50	50+	1.5–2.0	2.1–2.5	2.5+	a	b	c
Low-dose escalation											
1.0–5.1	8	11	2	1	—	—	—	—	—	—	—
6.0–12.0	7	11	1	1	1	—	1	1	3	1	—
High-dose escalation											
15.0	10	15	2	2	—	2	1	1	4	4	1
17.5	5	5	2	—	2	—	1	2	2	2	—
20.0	1	1	—	—	1	—	—	1	1	—	—

The onset, nadir, and duration of leukopenia varied, beginning as early as the 6th day and as late as the 26th day after the start of therapy. The median nadir was 14 days and, in most patients, recovery was complete by the 21st day. Thrombocytopenia tended to occur slightly later, with onset any time from the 10th to the 26th day; the median nadir was 21 days with recovery by the 28th day in all but one patient. The median duration of thrombocytopenia was 12 days.

Increasing BUN or creatinine levels or both were usually noted within 7–14 days after the start of therapy, with the median nadir at 21 days. In patients receiving several courses of the drug, the duration for recovery of BUN and creatinine levels was frequently quite prolonged, and in three patients treatment was discontinued because of the failure of these values to return to normal levels.

Toxic effects observed with the twice-weekly schedule are shown in tables 3 and 4. Again, at lower doses hematopoietic toxicity was minimal, but some increases were observed in BUN and creatinine levels. In most instances, it was felt that the increase in these values was related to other factors such as genitourinary infection, intestinal obstruction, or, in one patient, concomitant use of gentamycin. Renal toxicity occurred just as frequently at the 15-mg/m^2 dose level, and nausea and vomiting appeared to occur more frequently with the delayed dose regimen as treatment progressed. Because no real difference was observed, with the exception of gastrointestinal toxicity, the 5-day schedule was used for patients receiving dose levels greater than 15.0 mg/m^2.

Another factor to consider in the evaluation of toxicity is the possible cumulative effect of this drug on both the hematopoietic system

TABLE 3.—Evaluation of hematologic toxicity of *cis*-diamminedichloroplatinum on the twice-weekly schedule

Dose (mg/m^2)	No. of patients	No. of courses	Wbc (cells × $10^3/mm^3$) <3.5	<2.5	Platelets (cells × $10^4/mm^3$) <10	<5	Fall in hemoglobin level (>2 g)
Low-dose escalation							
1.0–5.1	7	9	0	0	1	0	1
6.0–11.0	6	7	0	0	0	0	0
High-dose escalation							
15.0	13	14	4	2	1	0	1

TABLE 4.—Evaluation of renal and gastrointestinal toxicity of *cis*-diamminedichloroplatinum on the twice-weekly schedule

Dose (mg/m^2)	No. of patients	No. of courses	Renal toxicity: BUN level (mg/100 ml) 25–35	36–50	51+	Creatinine level (mg/100 ml) 1.5–2.0	2.1–2.5	2.5+	Gastrointestinal toxicity a	b	c
Low-dose escalation											
1.0–5.1	7	9	1	1	2	2	1	—	1	1	0
6.0–11.0	6	7	1	1	1	1	—	1	1	—	—
High-dose escalation											
15.0	13	14	3	2	—	2	2	—	2	8	2

and the kidneys. *Cis*-diamminedichloroplatinum undoubtedly is an alkylating agent, and it is a recognized fact that most alkylating agents have the propensity for cumulative marrow toxicity. Since the drug is partly a heavy metal, even though tightly complexed, it is possible that cumulative renal toxicity may be due to persistence of the platinum ion in the renal tubules. Because a majority of the patients (50 of 57) had received prior chemotherapy, it was difficult to determine if this prior chemotherapy altered the degree or severity of hematopoietic toxicity. However, no greater toxicity was noted in the 16 patients who had received prior alkylating agent chemotherapy than was noted in the seven patients who had received only antimetabolites or no therapy.

Analyses of the data in patients who received two or more courses of either regimen at a dose level of 15 mg/m^2 or greater indicate that the toxicity may be cumulative. Even though only four patients received two or more courses with each regimen, there appeared to be increasing hematopoietic and possibly renal toxicity with prolonged exposure to the drug.

Other toxicity.—Marked ST-T wave changes developed on one patient's electrocardiogram during therapy, and congestive heart failure in this patient required digitalization. The patient had no prior history of heart disease. Tinnitus was observed in one patient. Audiometric studies were done prior to therapy and 4 weeks after therapy in eight patients who received 15 mg/m^2 for 5 days or higher doses. In four patients, there was approximately a 15% loss of hearing in the higher decibel range. Two patients were metal workers and already had significant loss of high decibel hearing; this was not accentuated by the therapy. Two other patients had no demonstrable deficit after therapy.

OBJECTIVE RESULTS

Many patients with a wide variety of neoplasms were treated at low dose levels and, thus, their courses of treatment could not be

considered therapeutic trials. However, of the 57 patients who completed one course of therapy, only five remissions were observed. The following malignant neoplasms were included: gastrointestinal, 18; lung,10; genitourinary, 6; lymphomas, 5; head and neck, 4; soft tissue sarcomas, 4; unknown primaries, 5; melanomas, 2; and one each of adrenal tumor, thymus tumor, and neuroblastoma.

The best objective remission was in the patient with malignant thymoma. His therapy was initiated at a dose level of 4 mg/m² for 5 days, and was subsequently escalated to 6 mg/m² for his second course and 10 mg/m² for his third and fourth courses. Because of azotemia, the dose was reduced to 5 mg/m² for six more courses. The patient was maintained in remission for 13 months, and then, because of increasing disease and azotemia, therapy was discontinued.

A transient 3-month decrease in hepatic metastases from a neuroblastoma was observed in a 3½-year-old girl. There was a 50% decrease in the size of the liver below the costal margin which lasted for 2 months after initial treatment with 5 mg/m² and subsequent treatment with 7.5 mg/m². Prolonged azotemia, however, prevented additional therapy.

Five patients with various non-Hodgkin's lymphomas were treated with doses of 15 mg/m² or greater. In three of these patients, reduction in the size of palpable nodes occurred and lasted for 4 months, 3 months, and 1 month, respectively.

Even though other objective responses were not observed, 50 patients, including those already described, had received intensive chemotherapy with a variety of agents, including cyclophosphamide, CCNU, adriamycin, actinomycin D, vincristine, NSC-45388, 5-fluorouracil, and combinations of various agents. Also, many of the patients had been treated with radiotherapy. However, only one of the patients who experienced an objective remission (the patient with the thymoma) had any subjective benefit from therapy.

DISCUSSION

Although *cis*-diamminedichloroplatinum has produced encouraging results in a variety of transplanted animal tumors, its use in humans may be limited by renal toxicity. It is possible, however, that many dose regimens other than those discussed here may prove beneficial. Also, adequate therapeutic trials with the dose regimen of 15 mg/m² need to be explored in patients not previously treated with many chemotherapeutic agents.

Since hematopoietic toxicity does not appear to be severe at the dose level of 15 mg/m², perhaps combinations of agents having different mechanisms of action should be considered in the future. Renal toxicity, which may be cumulative, appears to be the limiting factor in the prolonged utilization of this agent.

The mechanism of action of *cis*-diamminedichloroplatinum has not been definitely established, but it has been suggested that this compound and related compounds bind DNA in a manner similar to that of mechlorethamine.[6] More recently, Howle, Gale, and Smith reported that *cis*-diamminedichloroplatinum probably forms bonds with guanylate and uridylate in the nucleic acid spiral, and these bonds are probably dissimilar from the binding that is ascribed to mechlorethamine (4).

CONCLUSION

A phase I clinical evaluation of *cis*-diamminedichloroplatinum has been underway in the SOG for 2 years. We can conclude that a dose schedule of 15 mg/m² given daily for 5 days each month is acceptable to most patients. Both the hematopoietic system and the kidney function must be monitored carefully. Gastrointestinal toxicity, as manifested by nausea or vomiting or both, usually is controllable with this dose regimen. Further exploration in more patients and possibly evaluation of combinations of *cis*-diamminedichloroplatinum with other drugs are recommended.

LIST OF COMPOUNDS

Actinomycin D: NSC-3053
Adriamycin: NSC-123127
CCNU: NSC-79037; CAS reg. No. 13010-47-4; urea, 1-(2-chloroethyl)-3-cyclohexyl-1-nitroso-
***Cis*-diamminedichloroplatinum:** NSC-119875; CAS reg. No. 15663-27-1

[6] Talley R. *Cis*-platinum (II) diamminodichloride. Southwest Group Studies. National Cancer Institute Chemotherapy Program, Phase I–II Liaison Meeting, Mar 9, 1972, Bethesda, Md.

Cyclophosphamide: NSC-26271; CAS reg. No. 6055-19-2; 2*H*-1,3,2-oxazaphosphorine, 2-[bis(2-chloroethyl)-amino]tetrahydro-, 2-oxide, monohydrate

5-Fluorouracil: NSC-19893; CAS reg. No. 51-21-8; 5-FU

Mechlorethamine: NSC-762; CAS reg. No. 55-86-7; diethylamine, 2,2'-dichloro-*N*-methyl-, hydrochloride; nitrogen mustard

NSC-45388: CAS reg. No. 4342-03-4; 5-(3,3-dimethyl-1-triazeno)imidazole-4-carboxamide

Vincristine: NSC-67574; CAS reg. No. 2068-78-2; leurocristine, sulfate (1:1)

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Cell Survival and Repair of Plateau-Phase Cultures After Chemotherapy—Relevance to Tumor Therapy and to the In Vitro Screening of New Agents [1,2]

Brief Reports and Preliminary Communications

Gordon R. Ray, George M. Hahn, Malcolm A. Bagshaw, and Stephanie Kurkjian [3]

Density-inhibited cultured cells (plateau phase) and populations of solid tumor cells in vivo have many properties in common (1,2). Perhaps of greatest interest to the chemotherapist is the large fraction of out-of-cycle but potentially clonogenic cells contained in both of these groups. Because these cells are able to withstand relatively high doses of most chemotherapeutic agents, they may constitute the major obstacle to the successful treatment of many solid tumors. In previous studies it has been shown that noncycling cells in vitro or in vivo can repair potentially lethal lesions after X-irradiation (2–5). This repair is manifested as a dose-dependent increase in cell survival if the interval between X-irradiation and subculture in vitro or X-irradiation and tumor explant in vivo is increased. We have also demonstrated a similar phenomenon after chemotherapy in mice bearing the EMT6 mammary sarcoma (6). In this paper we describe in detail some of our in vitro findings (including repair) and discuss their relevance to chemotherapy for solid tumors. The close similarities between these findings and those obtained with the solid tumor system in vivo lead us to urge that consideration be given to including plateau-phase culture techniques in the screening of new drugs.

MATERIALS AND METHODS

Our cells (Chinese hamster ovary cells designated HA-1), culture conditions, and methods for obtaining plateau-phase cultures have been described in detail (1,7). Drug solutions were made up freshly prior to each experiment in Hanks' Balanced Salt solution (BSS) (solutions in media led to variable results usually traceable to the particular batch of serum used to supplement the medium). Cells (growing in monolayers) were exposed to the compounds in BSS at 37 C for 1 hour; subsequently, they were rinsed twice and either trypsinized immediately ("0" hour) or overlaid with fresh BSS, returned to the incubator for the repair period (usually 6 hours), and then trypsinized. The assay for cell survival was the Puck cloning technique. The exponentially growing cells used for comparison were treated and assayed in exactly the same manner.

RESULTS

Survival values for Chinese hamster cells ("0" hour) exposed to graded doses of mechlorethamine, 5-fluorouracil, and bleomycin [4] are shown in table 1, columns 2 and 3. These drugs illustrate three different situations. Mechlorethamine kills exponentially growing (cycling) cells and plateau-phase (noncycling) cells equally well. 5-Fluorouracil is more effective against actively cycling cells. These two agents follow the classification of Bruce et al (8). Bleomycin, however, preferentially kills noncycling cells. Only two other agents are reported to have similar properties: the methyl

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[4] Mechlorethamine: NSC-762; CAS reg. No. 55-86-7; diethylamine, 2,2'-dichloro-*N*-methyl-, hydrochloride; HN2; nitrogen mustard.

5-Fluorouracil: NSC-19893; CAS reg. No. 51-21-8; 5-FU.

Bleomycin: NSC-125066. Supplied by Cancer Therapy Evaluation (CTE), Division of Cancer Treatment (DCT), NCI.

TABLE 1.—Survival values for Chinese hamster cells

Drug	Concentration* (μg/ml)	Survival values† (%) "0" Hr: Exponentially growing cells	"0" Hr: Plateau-phase cells	After 6-hr repair period: Exponentially growing cells	After 6-hr repair period: Plateau-phase cells
5-Fluorouracil	PE	69	71	82	74
	5	64	95	100	100
	10	66	84	84	100
	15	66	84	83	94
	20	62	80	83	90‡
Bleomycin	PE	71	94	71	87
	25	NM	15	NM	44
	50	NM	12	26	32
	75	28	9.5	NM	24
	125	21	4.4	20	12
	250	20	2.4	18	9
Mechlorethamine	PE	63	71	65	71
	0.25	40	37	41	70‡
	0.5	21.5	20	32	45
	1.0	6.4	7.8	16	26
	2.0	1.0	0.56	1.5‡	2.7
	2.5	NM	0.24	NM	0.9

*PE = plating efficiency.
†NM = not measured.
‡Estimated by interpolation.

ester of methanesulfonic acid (9) and BCNU (10,11).[5] Of these drugs, only BCNU and bleomycin are useful for chemotherapy in man. In our hands, BCNU kills cycling and noncycling cells equally well.

Survival values for cells from cultures assayed after the 6-hour repair interval are listed in columns 4 and 5. In spite of the wide variation in the modes of action of these agents, repair (as evidenced by increased survival) occurs after exposure to each of them. Cells treated with mechlorethamine behave very much like X-irradiated cells: a significant amount of repair occurs only in plateau-phase cultures, and, even in these cultures, the increase in survival is not dramatic. After treatment with 5-fluorouracil, both exponentially growing and plateau-phase cells repair themselves. The data for bleomycin deserve special comment. The data shown in table 1 are from two early experiments. No repair was seen in two later experiments; however, it was discovered that at about that time the cultures had become contaminated with *Mycoplasma* organisms. Why such an infection should interfere with intracellular repair is not clear. In a final experiment, after the infection had been eliminated, repair was again observed.

DISCUSSION

The finding that bleomycin has the potential of preferentially killing noncycling cells (6)

[5] Methanesulfonic acid, methyl ester: NSC-50256; CAS reg. No. 66-27-3.

BCNU: NSC-409962; CAS reg. No. 154-93-8; urea, 1,3-bis(2-chloroethyl)-1-nitroso-. Supplied by CTE, DCT, NCI.

is by itself of some importance. Similar findings have recently been reported by Barranco et al (11). However, the efficient repair system of the resting cells quickly negates this advantage. The in vitro results shown in this paper have their exact counterpart in the EMT6 tumor system in vivo (6). When bleomycin doses higher than about 1–2 mg/kg are used in that system, additional cytotoxic action occurs only if the tumors are excised shortly after injection of the drug (at about 2 hours). If the tumors are permitted to remain in situ for 24 hours, the repair system can restore 99% of the cells to viability. (In reference 6 we suggested that the increased survival found in the cells from 24-hour tumors might be all or in part an artifact of the assay system and that perhaps cells killed by bleomycin can modify the survival of untreated [or not lethally injured] cells. Control experiments performed since then have shown this not to be the case; hence, the increase in the survival of the cells from the 24-hour tumors truly reflects some intracellular process.)

For both 5-fluorouracil and bleomycin, the in vitro data can be used to predict, at least qualitatively, the in vivo results. The EMT6 tumor has a growth fraction of approximately 50% (12). Thus, the tumor could be considered a composite of 50% actively cycling and 50% noncycling cells. Theoretical cell survival curves constructed for 50% exponentially growing and 50% plateau-phase cells have a biphasic shape; the extrapolation number of the terminal portion of the survival curve should be near the 50% survival level. These predictions, based strictly on in vitro results and knowledge of the tumor's growth fraction, are fulfilled to a rather surprising degree: the tumor cell population behaves (at least at initial treatment) as though it were made up of 50% cycling cells and 50% noncycling cells. Thus, the response of cells in solid tumors to chemotherapeutic agents is quite similar to the response of cells in vitro with respect to both initial cytotoxicity and postexposure repair.

This very close correspondence between the in vitro tumor model and in vivo findings leads us to suggest that plateau-phase cultures be used as part of any in vitro screening process. Furthermore, the importance of the repair process indicates that delayed subculture should be included as part of the screening protocol.

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Clinical Trial of Weekly Doses of Vinblastine (NSC-49842) Combined With Vincristine (NSC-67574) in Malignant Lymphomas and Other Neoplasms [1,2,3]

Brief Reports and Preliminary Communications

David C. Stolinsky, Gilbert J. Hum, Edwin M. Jacobs, Joel Solomon, and Joseph R. Bateman [4,5]

Since their introduction into clinical use in the early 1960s, vinblastine and vincristine have been shown to have significant therapeutic effect against lymphomas, leukemia, and other neoplasms (1). Although these agents are similar in chemical structure, differences in therapeutic and toxic effects have been demonstrated. The present study was designed to explore the therapeutic and toxic effects of various doses of vinblastine and vincristine used in combination.

METHODS

Patient Selection

Patients with measurable malignant tumors not amenable to surgical treatment or radiation therapy were eligible for the study. Patients who had been treated previously with cytotoxic agents or irradiation were eligible provided that at least 4 weeks had elapsed since the cessation of the therapy. No minimum performance status was required.

Clinical Evaluation

The following pretreatment studies were performed for each patient: history and physical examination; complete blood cell count (including platelet count); bone marrow aspiration; urinalysis; determination of serum urea nitrogen, uric acid, bilirubin, alkaline phosphatase, glutamic oxaloacetic transaminase, and calcium levels; serum protein electrophoresis; and chest and skeletal roentgenograms. Physical examination and complete blood cell counts were repeated weekly. Serum chemical determinations were repeated at intervals of 2–4 weeks. Roentgenograms were repeated when abnormalities were noted. In the assessment of response, the products of the perpendicular diameters of each measurable tumor were added together. A partial response was defined as at least a 50% reduction in the size of measurable tumors for at least 1 month. A complete response was defined as the disappearance of all signs of disease for at least 1 month.

Drug Administration

Both agents were administered weekly in escalating doses as shown in table 1. They were prepared and drawn up in separate syringes. Dose escalation was interrupted at the first sign of toxic effects. If the disease failed to

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[3] Vinblastine: CAS reg. No. 6449-03-2; vincaleukoblastine, sulfate (1:1), monohydrate.

Vincristine: CAS reg. No. 2068-78-2; leurocristine, sulfate (1:1).

The compounds used in this study were supplied by Cancer Therapy Evaluation, Division of Cancer Treatment, NCI, and by Eli Lilly & Co.

[4] The following members of the Western Cooperative Cancer Chemotherapy Group participated in this study: *J. Bateman, D. Bogdon, G. Hum, L. Irwin, and D. Stolinsky*, University of Southern California and John Wesley Hospital, Los Angeles; *E. Jacobs and D. Wood*, University of California, San Francisco; *J. Solomon*, City of Hope Medical Center, Duarte, Calif; *R. Hestorff and A. Stevens*, University of Washington, Seattle; *R. Pugh*, Ellis Fischel State Cancer Hospital, Columbia, Mo; *J. Braunwald*, Kaiser Foundation Hospital, Los Angeles, Calif; *H. Kimball*, Memorial Hospital, Phoenix, Ariz; *R. Reynolds*, David Grant US Air Force Hospital, Travis Air Force Base, Calif; *R. Goldman*, University of Oregon, Portland.

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TABLE 1.—Dose schedule for weekly treatment with vinblastine plus vincristine

Drug	Week:	Dose (mg/kg) 1	2	3	4	5	6	7	8
Vinblastine		0.070	0.084	0.100	0.120	0.144	0.158	0.174	0.191
Vincristine		0.008	0.010	0.012	0.014	0.017	0.020	0.024	0.026

TABLE 2.—Experience with weekly treatment of various neoplasms with vinblastine plus vincristine

Neoplasm (type or site)	Total No. of patients	No. of patients in the study for 4 wks	No. of patients who had— Partial response*	Complete response*
Lymphoma				
Lymphocytic	16	14	4	
Histiocytic	7	7	3	1
Hodgkin's	12	10	5	2
Sarcoma				
Rhabdomyosarcoma	6	4	1	
Mesenchymoma	1	1		
Leiomyosarcoma	1	1		
Anaplastic	1			
Osteosarcoma	1			
Other				
Lymphosarcoma cell leukemia	3	1		
Myeloma	1			
Parotid gland	1	1		
Nasopharynx	2	2		
Esophagus	1			
Colon	2	2		
Pancreas	1	1		
Breast	2			
Cervix	1	1		
Uterus	2	1		
Ovary	2	2		
Bladder	1	1		
Kidney	1	1		
Thymoma	1	1		
Unknown	2	1		
Total	68	52	13	3

*See text for definition of response.

TABLE 3.—Toxic effects of weekly treatment with vinblastine plus vincristine in 66 patients with various neoplasms

Toxic effect	Severity of effect				Total
	Mild	Moderate	Severe	Extreme	
	No. of patients				
Leukopenia*	13	20	11	4	48
Thrombocytopenia*	9	3	2	2	16
Anemia	2				2
Nausea, vomiting	2	4			6
Diarrhea		1			1
Constipation	1	3	1		5
Stomatitis	1				1
Paresthesia	17	6	6		29
Weakness	2	3	6		11
Depression	2	1			3
Alopecia	5	1			6

*See text for explanation of severity of leukopenia and thrombocytopenia.

respond after four weekly injections, therapy was discontinued. If the disease responded, therapy was continued as tolerated. All patients were placed on a laxative regimen. The strength of wrist extensors and ankle dorsiflexors was measured objectively to detect early motor neuropathy.

RESULTS

Sixty-eight patients were studied. Sixty-six patients could be observed for 1 week after the first dose and were considered evaluable. Most patients did not progress beyond the fifth dose level because toxic effects occurred.

Response

(*See* table 2). Responses began at a median of 2 weeks of therapy (range, 1–8 weeks), at a median dose of vinblastine of 0.153 mg/kg (range, 0.064–0.802 mg/kg), and at a median dose of vincristine of 0.018 mg/kg (range, 0.008–0.111 mg/kg). Responses lasted a median of 9 months (range, 1.5–88 months). Of the 16 patients who responded to therapy, ten had received irradiation prior to the study, nine had received an alkylating agent, three had received vinblastine, and four had received no prior therapy.

Toxic Effects

(*See* table 3.) Leukopenia was classified as mild (3000–3900 cells/mm^3), moderate (2000–2900 cells/mm^3), severe (1000–1900 cells/mm^3), or extreme (< 1000 cells/mm^3). Thrombocytopenia was classified as mild (100,000–149,000 platelets/mm^3), moderate (60,000–99,000 platelets/mm^3), severe (35,000–59,000 platelets/mm^3), or extreme (< 35,000 platelets/mm^3). Leukopenia occurred at a median vinblastine dose of 0.212 mg/kg (range, 0.083–0.756 mg/kg) and at a median vincristine dose of 0.025 mg/kg (range, 0.010–0.086 mg/kg). No deaths were attributable to therapy.

Toxic neuropathy was noted; occasionally it was severe. Neuropathy occurred at a median vinblastine dose of 0.303 mg/kg (range, 0.083–0.756 mg/kg) and at a median vincristine dose of 0.036 mg/kg (range, 0.010–0.100 mg/kg). Paresthesia of the fingers usually preceded weakness, but some patients lost 25% of their extensor strength with minimal sensory symptoms. Thus, objective measurement of extensor strength was useful in detecting significant neuropathy. The vincristine dose was reduced when mild paresthesia occurred; the drug was withheld when moderate paresthesia or a 25% decrease in extensor strength occurred.

DISCUSSION

The mechanisms of action of the vinca alkaloids have been extensively studied. Mitotic arrest through binding of the agents to a precursor of the spindle has been described (2,3). This may occur in S phase, with disorganization of the spindle becoming obvious only when the next mitosis is attempted (4). In-

hibition of the DNA-dependent RNA polymerase system has also been described (3). Although these actions are common to both vinblastine and vincristine, some differences between them have become apparent. Mammalian cells are sensitive to vinblastine during the G_1 and S phases, but they are sensitive to vincristine only during S phase. Thus, a greater effect on rapidly dividing cells might be predicted for vincristine (4). The greater potential of vincristine to produce neuropathy may be related to its inhibition of acetate incorporation into phospholipid, a significant component of myelin (5).

In some animal tumor systems, cross resistance between vinblastine and vincristine has been demonstrated (6). Additive effects have also been described (7,8). Potentiation of the effects of other vinca alkaloids has been described only for vinrosidine,[6] which is not in clinical use (8). Our experience is that patients with lymphomas resistant to vinblastine may still respond to vincristine and vice versa. An additional rationale for combining these agents relates to their differing toxic effects. Myelosuppression is a major effect of vinblastine, but it is seen less often with vincristine. The reverse is true for neuropathy. The only previous report of treatment with the combination of vinblastine and vincristine involved a trial in children (9). In that study, complete remissions were obtained in seven of 22 patients with lymphoblastic leukemia. Many of the patients were resistant to vincristine. Since vinblastine has been ineffective when given alone in the treatment of this disease (1), it would appear that the effects were not simply additive.

In the present study, responses were obtained in patients with lymphomas of the three major types. No direct comparison can be made between the response rates obtained in the present study and the response rates obtained with the same agents used singly or the response rates obtained with other combinations of agents. For such a comparison, a randomized study with control of variables such as age, duration and extent of disease, histologic subclassification, and prior therapy would be required. Too few patients resistant to vinblastine were treated to allow us to make any conclusions as to whether the therapeutic effects observed were additive; however, the response of one patient was better than what might have been anticipated for vincristine alone. The toxic effects produced by the combination of vinblastine and vincristine were similar to those produced by the agents used singly, and no aggravation of myelosuppression or neuropathy was apparent. The laxative regimen appeared to be helpful in preventing obstipation, and the objective muscle testing program was helpful in detecting early motor neuropathy.

Treatment with the combination of vinblastine and vincristine has been shown to be clinically feasible. The addition of other effective agents to this combination might be of interest in the therapy for lymphoma in man.

[6] Vinrosidine sulfate: CAS reg. No. 1405–55–6.

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Vinblastine (NSC-49842) Plus Vincristine (NSC-67574) Given in Intensive 3-Day Courses as Therapy for Lymphomas, Sarcomas, and Other Neoplasms [1,2,3]

Brief Reports and Preliminary Communications

David C. Stolinsky, Donald L. Bogdon, Reginald P. Pugh, Jack Braunwald, Robert Hestorff, and Joseph R. Bateman [4,5]

Vinblastine and vincristine are active against a variety of neoplasms (1). In a previous report (2), we examined the effects of these agents given together in weekly injections. The present study was conceived in an effort to improve the response rate by giving the same agents on a more intensive schedule.

METHODS

Patient Selection

Patients with measurable malignant tumors not amenable to surgical treatment or radiation therapy were eligible for the study. Patients who had been treated previously with cytotoxic agents or irradiation were eligible provided that at least 4 weeks had elapsed since cessation of the therapy. Patients were classified as "poor risk" under any of the following circumstances: (*a*) if the leukocyte count was less than 5000 cells/mm^3; (*b*) if there was a loss in body weight of more than 40%; (*c*) if the serum urea nitrogen value was more than 30 mg/100 ml; (*d*) if the tumor had invaded the marrow; or (*e*) if there was a history of irradiation of a significant portion of the marrow or extensive therapy with an alkylating agent. Thirty-five of the 124 patients studied had received irradiation prior to the study, 27 had received an alkylating agent, 27 had received other therapy, and 35 had not been treated.

Clinical Evaluation

The following pretreatment studies were performed for each patient: history and physical examination; complete blood cell count including platelet count; bone marrow aspiration; urinalysis; determination of serum urea nitrogen, uric acid, bilirubin, alkaline phosphatase, glutamic oxaloacetic transaminase, and calcium levels; and chest and skeletal roentgenograms. Physical examination and complete blood cell counts were repeated weekly. Serum chemical determinations were repeated every 2–4 weeks. Roentgenograms were repeated when abnormalities were noted. In the assessment of response, the products of the perpendicular diameters of each measurable tumor were added together.

Drug Administration

The drugs were drawn up in separate syringes. Both agents were given daily for 3 successive days. The initial daily dose for "good-risk" patients was 0.120 mg/kg of vinblastine and 0.014 mg/kg of vincristine; for "poor-risk" patients, the initial daily dose was 0.100 mg/kg of vinblastine and 0.012 mg/kg of vincristine. Because of the toxic effects observed, the dose for "good-risk" patients was reduced to 0.084 mg/kg of vinblastine and 0.010 mg/kg of vin-

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[3] Vinblastine: CAS reg. No. 6449–03–2; vincaleukoblastine, sulfate (1:1), monohydrate.

Vincristine: CAS reg. No. 2068–78–2; leurocristine, sulfate (1:1).

The compounds used in this study were supplied by Cancer Therapy Evaluation, Division of Cancer Treatment, NCI, and by Eli Lilly & Co.

[4] The following members of the Western Cooperative Cancer Chemotherapy Group participated in this study: *R. Pugh*, Ellis Fischel State Cancer Hospital, Columbia, Mo; *J. Bateman, D. Bogdon, G. Hum, L. Irwin, and D. Stolinsky*, University of Southern California and John Wesley Hospital, Los Angeles; *J. Braunwald and M. Yettra*, Southern California Permanente Medical Group, Los Angeles; *R. Hestorff and A. Stevens*, University of Washington, Seattle; *J. Solomon*, City of Hope Medical Center, Duarte, Calif; *H. Kimball*, Memorial Hospital, Phoenix, Ariz.

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cristine. "Poor-risk" patients were treated with 0.070 mg/kg of vinblastine and 0.008 mg/kg of vincristine. After a 3-day course of treatment with both agents, no therapy was given for at least 18 days (or longer if more time was required for toxic effects to clear). The 3-day course of therapy was then repeated, with the dose level determined by the patient's risk status at that time. If the disease responded, courses were repeated every 3 weeks as tolerated. All patients were treated with laxatives.

RESULTS

Of the 124 patients studied, 112 could be observed for 1 week after the first dose; these patients were evaluated for response and drug toxicity. A partial response was defined as at least a 50% reduction in the size of measurable tumors for at least 1 month. A complete response was defined as the disappearance of all signs of disease for at least 1 month.

Response

(*See* table 1.) Responses began at a median of 10 days after the start of therapy (range, 4–40 days), at a median vinblastine dose of 0.252 mg/kg (range, 0.147–0.376 mg/kg), and at a median vincristine dose of 0.030 mg/kg (range, 0.018–0.043 mg/kg). Responses lasted a median of 2 months (range, 1–68 months).

TABLE 1.—Experience with daily treatment of various neoplasms with vinblastine plus vincristine

Neoplasm (type or site)	Total No. of patients	No. of patients in the study for 4 wks	No. of patients who had—	
			Partial response*	Complete response*
Lymphoma				
Lymphocytic	10	7	1	2
Histiocytic	11	10	2	1
Hodgkin's disease	4	3	2	
Sarcoma				
Rhabdomyosarcoma	9	6		1
Leiomyosarcoma	7	5		
Fibrosarcoma	3	2		
Liposarcoma	2	1	1	
Myxosarcoma	1	1		
Hemangiosarcoma	1	0		
Osteosarcoma	3	2		
Chondrosarcoma	1	1		
Ewing's sarcoma	1	1		
Anaplastic	3	2	1	
Other				
Salivary gland	1	1		
Lymphoepithelioma	1	1	1	
Stomach	1	1		
Colon	1	0		
Hepatoma	1	1		
Pancreas	3	3		
Mesothelioma	4	1		
Lung, oat cell	6	4	2	

TABLE 1.—Experience with daily treatment of various neoplasms with vinblastine plus vincristine—Continued

Neoplasm (type or site)	Total No. of patients	No. of patients in the study for 4 wks	No. of patients who had—	
			Partial response*	Complete response*
Lung, other	15	9		
Melanoma	5	3		
Breast	2	1	1	
Cervix	3	3		
Uterus	3	1		
Ovary	2	1		
Prostate	2	1		
Testis	1	1		
Urethra	1	1		
Kidney	9	6	1	
Neuroblastoma	1	0		
Unknown	6	3		
Total	124	83	12	4

* *See* text for definition of response.

Toxic Effects

(*See* table 2.) Leukopenia was classified as mild (3000–3900 cells/mm³), moderate (2000–2900 cells/mm³), severe (1000–1900 cells/mm³), or extreme (<1000 cells/mm³). Thrombocytopenia was classified as mild (100,000–149,000 platelets/mm³), moderate (60,000–99,000 platelets/mm³), severe (35,000–59,000 platelets/mm³), or extreme (< 35,000 platelets/mm³). Leukopenia occurred at a median vinblastine dose of 0.248 mg/kg and at a median vincristine dose of 0.030 mg/kg. Three patients died during a period of leukopenia. Neuropathy occurred at a median vinblastine dose of 0.302 mg/kg and at a median vincristine dose of 0.036 mg/kg. Toxic neuropathy was severe in six patients.

TABLE 2.—Toxic effects of daily treatment with vinblastine plus vincristine in 112 patients with various neoplasms

Toxic effect	Severity of effect				Total
	Mild	Moderate	Severe	Extreme	
	No. of patients:				
Leukopenia *	21	20	26	20	87
Thrombocytopenia *	22	7	4	1	34
Gastrointestinal disturbances (nausea, vomiting, constipation)	36	9	8		53
Neuropathy	27	28	6		61
Alopecia	56				56

* *See* text for explanation of severity of leukopenia and thrombocytopenia.

DISCUSSION

The rationale for combining vinblastine and vincristine included differences in their modes of action and therapeutic and toxic effects. In a previous report (2), we described the results obtained with these agents given together weekly. Significant responses were obtained in 43% of the patients with lymphoma, most of whom had received prior therapy with other agents. The toxic effects of the weekly schedule were tolerable, with four instances of extreme leukopenia in 66 patients. In the present study, extreme leukopenia was observed in 20 of the 112 patients that could be evaluated for response and drug toxicity. It is possible that the three deaths in the group of patients treated with the more intensive schedule were caused by the drugs. Although a direct comparison of the response rates and toxic effects of the weekly and daily regimens would require a randomized study, the available evidence supports the view that the daily schedule is more toxic without yielding better response rates. Moreover, the median duration of remission was 9 months with the weekly schedule and 2 months with the daily schedule. Therefore, we decided to abandon further trial of treatment with the vinca alkaloids given on a daily schedule.

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Cytosine Arabinoside (NSC-63878) and Daunorubicin (NSC-83142) Therapy in Acute Nonlymphocytic Leukemia [1,2,3]

Jerome W. Yates, H. James Wallace, Jr., Rose Ruth Ellison, and James F. Holland [4]

Early destruction of leukemic infiltration in the induction phase of treatment may reduce the duration of time most hazardous for infection as well as the total period of necessary hospitalization. Daunorubicin produces rapid bone marrow depression, and when administered as a single agent it is active in acute myelocytic leukemia, producing 43% remissions after a 5-day course (1). At the effective dose, however, the mortality and morbidity rates are high. The combination of daunorubicin and cytosine arabinoside appears to be quite active in the treatment of acute myelocytic leukemia. Remission rates of 79%, 60%, 60%, and 40% have been previously reported (2–5).

In previous experience, two daily doses of daunorubicin (45 mg/m²/day) and a 5-day continuous intravenous (iv) infusion of cytosine arabinoside (100 mg/m²/day) have produced subtotal bone marrow suppression after a single course, suggesting that an extension in the administration time of these drugs might produce adequate suppression after a single course.[5] The present study was undertaken to intensify the effects of cytosine arabinoside and daunorubicin thereby producing rapid destruction of leukemic cells. Such an effect might attain more remissions and earlier discharges from the hospital.

METHODS

All adult patients admitted to Roswell Park Memorial Institute with acute nonlymphocytic leukemia who had received no prior daunorubicin therapy and who were not in remission were studied during a 6-month period. All previously treated patients demonstrated M_3 or M_4 bone marrows, and seven of seven patients previously treated with cytosine arabinoside had achieved earlier complete remissions. They received an induction program that included cytosine arabinoside, 100 mg/m²/day via a continuous iv infusion for 7 days, and daunorubicin, 45 mg/m² given directly iv on Days 1, 2, and 3.

Throughout the induction phase, patients were assessed at least weekly for evidence of renal, cardiac, or hepatic malfunction. Bone marrow specimens were obtained prior to the administration of chemotherapy and then weekly thereafter until patient response was determined. Leukocyte, platelet, and hemoglobin levels were determined at least four times each week. Protective isolation (if available) and platelet and leukocyte transfusions were used for patient support. Patients were defined as having complete remission when there were fewer than 5% blast cells or other leukemic cells in a normally cellular bone marrow with evidence of normal erythropoiesis and granulopoiesis, and when the peripheral red blood cell, platelet, and granulocyte counts were restored to normal (6). Patients were defined as having partial remission when

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[2] Supported by grants CA-2599, CA-5834, and CA-10044 from the National Cancer Institute (NCI), National Institutes of Health, Public Health Service.

[3] Cytosine arabinoside: CAS reg. No. 69-74-9; cytosine, 1-β-D-arabinofuranosyl-, monohydrochloride; ara-C. Daunorubicin: CAS reg. No. 2341-50-6; daunomycin (NSC-82151).

Both drugs were supplied by Cancer Therapy Evaluation, Division of Cancer Treatment, NCI.

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[5] Hoagland HC, and Kyle R. Comparison of intermittent Ara C + cyclophosphamide with Ara C + DNR in induction and maintenance of untreated acute myelocytic leukemia. Minutes of the Acute Leukemia Group B meeting, July 29, 1972, p 22.

TABLE 1.—Patient characteristics

Patient No.	Sex, age	Diagnosis*	Previous antileukemic therapy†	Previous cytosine arabinoside†	Previous daunorubicin†	Initial hematologic status: White blood cell count (cells/mm³)	Initial hematologic status: Platelet count (cells/mm³)	Initial hematologic status: Infection§	Results‡	Remission duration (wks)
1	M, 29	AML	−	−	−	118,600	27,500	−	CR	14
2**	M, 58	AML	−	−	−	15,300	15,000	−	CR	20+
3**	M, 17	AML	−	−	−	127,500	5,000	−	CR	14
4**	M, 58	AMML	−	−	−	36,200	40,000	−	CR	24+
5	M, 17	APL	−	−	−	53,200	17,500	+	CR	8+
6	M, 78	AML	−	−	−	7,400	47,500	−	Death (bronchopneumonia)	
7	M, 67	AEL	−	−	−	1,800	35,000	−	Death (aortic stenosis, acute pulmonary edema)	
8	M, 76	AML	−	−	−	12,700	12,500	−	Death (arterial sclerotic heart disease; pulmonary edema)	
9	F, 33	APCL	−	−	−	2,650	85,000	+	CR	24+
10	F, 55	AML	+	+	−	8,400	537,500	−	PR	11
11	F, 52	AMML	+	+	−	3,150	152,500	−	PR	6
12	F, 50	AML	+	+	−	3,100	205,000	−	CR	14+
13	F, 26	AML	+	+	−	2,200	2,500	−	PR	6
14	M, 57	AML	+	+	−	2,850	500	+	NR	
15	M, 34	AMML	+	+	−	2,300	270,000	−	CR	8+
16††	M, 43	AEL	+	−	−	4,900	30,000	+	Death (bronchopneumonia)	
17	M, 60	AML	+	+	−	5,600	12,500	−	Death (lobar pneumonia)	

*AML = acute myelocytic leukemia; AMML = acute myelomonocytic leukemia; APL = acute promyelocytic leukemia; AEL = acute erythroleukemia; APCL = acute plasma cell leukemia.
†+ = Therapy received; − = no therapy received.
‡CR = complete remission; PR = partial remission; NR = no remission.
§+ = Infection acquired; − = no infection acquired.
**Patients receiving a second course of chemotherapy.
††Patient admitted with severe bilateral pneumonia.

there were 5%–25% leukemic cells in the bone marrow.

RESULTS

Sixteen patients with acute myelocytic leukemia and one patient with acute plasma cell leukemia were studied (*see* table 1). No patient was excluded from this analysis. The group was composed of 12 men and five women ranging from 17 to 78 years of age with a median of 53 years.

There were five complete remissions among eight patients with previously untreated acute myelocytic leukemia. A complete remission was achieved in the previously untreated patient who had acute plasma cell leukemia. There were two complete and three partial remissions among the eight patients who had received previous antileukemic therapy. There were five deaths, including one of the 13 patients who were below the age of 60, and four of the four patients who were 60 and above. Death occurred in these study patients on Days 5, 13, 16, 17, and 19 respectively. In two of these five patients, residual bone marrow leukemic activity was evident at the time of death.

In our group of patients, bone marrow hypoplasia and recovery occurred earlier than for a similar group of patients previously treated at Roswell Park Memorial Institute. In this previous study group, patients achieving remission with combinations of cytosine arabinoside and 6-thioguanine[6] given once daily (Acute Leukemia Group B protocol 7021) spent a median of 53 days in the hospital, whereas our patients who attained remission had a median of 35 days of hospitalization. At least a 2-week reduction in the median and mean hospitalization time is noted; however, the differences are not statistically significant.

The toxic effects noted were nausea, vomiting, mouth ulcerations, alopecia, and myelosuppression. Severe bronchopneumonia was the cause of death in three of the five patients dying during the study. Two patients were isolated in laminar air-flow rooms and one of these patients demonstrated severe bilateral pneumonitis prior to the initiation of isolation or chemotherapy.

DISCUSSION

The remission rate for both previously treated and previously untreated acute myelocytic leukemia patients was 63% with five of eight patients responding in each group. The three patients failing to achieve a complete remission in the previously untreated group were elderly. The one patient with acute plasma cell leukemia achieved complete remission status. Although seven of eight of the patients previously treated had received cytosine arabinoside in combination with other chemotherapeutic agents, there were still two complete remissions and three partial remissions.

Two earlier studies employing a combination of cytosine arabinoside and daunorubicin reported a mean daunorubicin induction dose of 360 mg and 360 mg/m² respectively (2,5). Our patients achieving remission status received an average of only 160 mg/m² of daunorubicin. The significant cardiac toxicity associated with daunorubicin, especially when given in large doses, suggests the desirability of reducing this drug to its smallest dose which is still effective (7).

Using our schedule of daunorubicin and continuous cytosine arabinoside, remission was achieved in 65% of the patients with a substantial reduction in hospitalization time. This program rapidly produces aplastic bone marrows without excessive toxicity. This pilot study has served as the basis for a further comparative evaluation which has already begun.

[6] 6-Thioguanine: NSC-752; CAS reg. No. 1832-72-0; purine-6-thiol, 2-amino-.

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Aeromonas Hydrophila Peritonitis [1]

Reisuke Saito [2,3] and Suzanne Schick [2]

Infectious diseases with *Aeromonas* are rare. The present report describes the clinical features and autopsy findings of a patient in whom *Aeromonas hydrophila* peritonitis developed after postoperative chemotherapy and irradiation were given for papillary serous cystadenocarcinoma of the ovaries. In this case, it is likely that *Aeromonas* peritonitis developed as an opportunistic infection secondary to chemotherapy and irradiation.

CASE REPORT

In October 1968, a 42-year-old white woman who had had menometrorrhagia for 12 years was admitted to the Magee Womens Hospital because of a uterine prolapse and a bilateral ovarian tumor. Hysterectomy, bilateral salpingo-oophorectomy, and partial omentectomy were performed for papillary serous cystadenocarcinoma of the ovaries. During the operation, seeding of tumor to the peritoneum was found. Postoperatively, she received approximately 5200 rads of deep irradiation to the abdomen and 65 mg of triethylenethiophosphoramide (thioTEPA) intravenously in four divided doses.[4] In April 1969, ascites developed, and a cytologic study of the ascitic fluid revealed malignant cells. Ninety mg of thioTEPA was given intraperitoneally in two divided doses following paracentesis. In July 1969, the patient was re-admitted because of the development of nausea and vomiting of 1 week's duration. She also experienced decreased urine output, abdominal pain, constipation, and severe epigastric burning.

Upon physical examination, the patient's blood pressure was 130 mm Hg systolic and 94 mm Hg diastolic, her pulse rate was 120 per minute, her respirations were 22 per minute, and her temperature was normal. Positive findings included massive ascites with a bilaterally elevated diaphragm, abdominal tenderness to palpation, and hypoactive bowel sounds.

The results of laboratory examinations were within normal limits except for a urinalysis which disclosed a trace of albumin, and many epithelial cells and a small number of bacteria in the sediment. A chest roentgenogram showed moderate left pleural effusion with compression of the left lung. The abdominal film disclosed ascites and considerable distention of the small intestine with fluid levels.

After admission, the patient continued to have nausea, vomiting, and epigastric burning in association with dyspnea which required frequent bilateral thoracocentesis. Her condition remained unchanged until 3 weeks after admission, when severe hypotension developed. The white blood cell count at that time was 11,600 cells/mm^3. She died shortly thereafter. Her temperature ranged between 36.0 and 37.2 C throughout her hospital course; no antibiotics were administered.

AUTOPSY FINDINGS

At the necropsy performed 16 hours after death, the peritoneal cavity contained 7.5 liters of serosanguineous fluid. The peritoneum was diffusely covered by a thin layer of yellow fibrinous exudate, beneath which were diffuse disseminations of tumor. There were marked fibrous adhesions, and kinking and stenosis of the terminal ileum with severe dilatation of the jejunum and ileum. No perforation was found in the intestinal tract. There was severe perivesical fibrosis with bilateral hydro-ureter and hydronephrosis. The kidneys also showed

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[4] ThioTEPA: NSC-6396; CAS reg. No. 52-24-4; phosphine sulfide, tris(1-aziridinyl)-.

acute tubular necrosis. The right and left pleural cavities contained 3 and 1.2 liters of serosanguineous fluid respectively, without evidence of acute inflammation or tumor metastasis. The lung showed severe congestion with focal hemorrhage and atelectasis. Other findings included acute splenitis and fatty metamorphosis of the liver.

The postmortem culture of heart blood grew *Escherichia coli* and *Proteus mirabilis*

The culture of ascitic fluid on a 5% rabbit blood agar plate yielded unusual green colonies with beta hemolysis. After biologic studies the organism was identified as *Aeromonas hydrophila.*

DISCUSSION

The genus *Aeromonas* is facultatively anaerobic, asporogenous, polar-flagellated, and gram-negative rods which ferment a number of carbohydrates with production of acids and often gas in peptone media (1) and give a positive oxidase reaction (2). These organisms are commonly found in soil and water and cause diseases in cold-blooded animals such as edema disease in carp and red leg in frogs (1). The organisms may easily be mistaken for members of the family *Enterobacteriaceae* because of a close resemblance in their growth pattern in routine culture; nonetheless, differentiation can readily be made by their positive oxidase reaction and polar flagellation (2).

Among four subspecies—*A. hydrophila, A. punctata, A. salmonicida,* and *A. proteolytica*—only *A. hydrophila* has been isolated from human sources such as bile, blood, nasal secretion, peritoneal fluid, pus, sputum, stool, throat swab, and urine (3) since as early as 1937 (4).

The first report of human *Aeromonas* infection by Hill et al in 1954 (5) called attention to the human pathogenicity of *A. hydrophila* and, since then, cases have occasionally been reported. Helm and Stille (6) collected 26 instances of human *Aeromonas* infection from the literature up to 1970 and added two cases of acute enteritis. In addition, Wassum (7) reported a case of acute *Aeromonas* enteritis in 1967 and Naumann et al (8) described a retroperitoneal abscess in 1969.

These 30 reported cases consist of 11 cases of sepsis (including one of peritonitis), eight of enteritis, three of tonsillitis, three of wound infection (including one with osteomyelitis), and single examples of pleural empyema, iritis, aspiration pneumonia, cholangitis, and retroperitoneal abscess.

The case of *Aeromonas* peritonitis described by Conn (9) was observed in a patient with Laennec's cirrhosis in association with *Aeromonas* septicemia. In 1968, von Graevenitz and Mensch (3) reported 28 instances in which *A. hydrophila* was isolated from various sites of the body. In two instances, the organism was cultured from peritoneal fluid with other pathogens such as a sequela of ruptured appendicitis.

The pathogenesis of *Aeromonas* infection often remains unknown. However, von Graevenitz and Mensch (3) report that all available evidence suggests that the gastrointestinal tract is the main habitat of human *Aeromonas* strains and that these organisms are waterborne.

Infective peritonitis is a rare complication of peritoneal tumor seeding. The absence of an intestinal perforation suggests that contamination of ascitic fluid occurred by transmural migration of the organism. In support of this was the probable damage of the intestinal wall both by increased luminal pressure due to stenosis and peritoneal tumor implants. In the present case, it can be presumed that the widely disseminated tumor and the administration of chemotherapy and irradiation were factors in lowering the patient's resistance, permitting an opportunistic infection.

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Treatment of Advanced Squamous Cell Carcinoma of the Gastrointestinal Tract With Bleomycin (NSC-125066) [1,2,3]

Mario Ravry, Charles G. Moertel, Allan J. Schutt, R. G. Hahn, and R. J. Reitemeier [4]

Bleomycin is an antibiotic derived from the fermentation products of *Streptomyces verticillus*. It has shown activity in several mouse tumor systems including Ehrlich ascites and Lewis lung carcinomas. As an antineoplastic agent, bleomycin is unique because of its preferential concentration in squamous epithelium and lung tissues and because of the rarity of significant hematologic toxicity.[5] A recent review of cumulative clinical data by Blum et al (1) shows that objective responses have been achieved with bleomycin in patients with squamous cell carcinoma of the head and neck and male and female genitalia, as well as in patients with lymphoma and testicular neoplasms. Reports concerning squamous cell carcinoma of the esophagus have been conflicting. Objective responses were achieved in 54% of the 22 patients evaluated in European studies but in only 8% of the 24 patients treated in the United States. To further evaluate the usefulness of bleomycin therapy we have conducted a clinical study of this agent in patients with advanced gastrointestinal squamous cell carcinoma, particularly that of esophageal origin.

MATERIALS AND METHODS

Patient Selection

Sixteen patients with advanced and metastatic squamous cell carcinoma of the gastrointestinal tract were selected for study. The origin of the primary cancer was esophageal in 14 patients and pancreatic in two patients. All had positive histologic confirmation of unresectable cancer, and all had areas of known malignant disease measurable with a ruler or calipers to serve as objective indicators of response to therapy. All patients were ambulatory and maintaining a reasonable state of nutrition. Fourteen patients had received no cytotoxic drugs prior to the study. One patient had been treated with chromomycin A_3 and one patient had been treated with both methotrexate and vincristine.[6] Nine patients had received roentgen therapy. None of the patients had received surgical treatment, chemotherapy, or radiotherapy within 1 month of entry into the study.

Treatment Procedure

Bleomycin was administered by direct intravenous injection at a dose of 20 mg/m^2 on a daily basis until a total dose of 280 mg had been delivered. Pulse rate, body temperature, and blood pressure were measured and recorded daily. The following laboratory studies were performed immediately before treatment: determination of hemoglobin value; white blood cell count (wbc) and differential;

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[2] Supported in part by contract NIH-70-2066 from the Division of Cancer Treatment (DCT), National Cancer Institute (NCI), National Institutes of Health.

[3] Bleomycin was supplied by Cancer Therapy Evaluation, DCT, NCI.

[4] Division of Gastroenterology and Internal Medicine (Drs. Ravry, Moertel, Schutt, and Reitemeier) and Division of Clinical Oncology and Internal Medicine (Dr. Hahn), Mayo Clinic and Mayo Foundation, Rochester, Minn.

[5] Rosenbaum C, and Carter SK. Clinical brochure: bleomycin (NSC 125066, "Bleo", BLM). Prepared by the NCI, Bethesda, Md, 1970, 29 pp.

[6] Chromomycin A_3: NSC-58514; CAS reg. No. 7059-24-7.

Methotrexate: NSC-740; CAS reg. No. 59-05-2; glutamic acid, *N*-[*p*-[[(2,4-diamino-6-pteridinyl)methyl]-methylamino]benzoyl]-.

Vincristine: NSC-67574; CAS reg. No. 2068-78-2; leurocristine, sulfate (1:1).

platelet count; determination of serum creatinine, bilirubin, alkaline phosphatase, and glutamic oxaloacetic transaminase (SGOT) values; and a chest roentgenogram. Wbc counts were repeated twice weekly and platelet counts were repeated once weekly for a total of 4 weeks after treatment. Four weeks after the completion of bleomycin therapy, the pretreatment laboratory studies were repeated, and objective response, symptomatic response, and general clinical status were evaluated.

RESULTS

Toxicity

The significant toxic reactions to bleomycin therapy are listed in table 1. Stomatitis and dermatitis were frequent but most commonly of only mild to moderate severity. The dermatitis was often heralded by soreness and swelling of the fingertips followed by redness and thickening of the skin over the hands and elbows, frequently with vesiculation. Pressure points such as the knuckles and olecranon process were most severely affected. Similar but milder changes were observed in the feet of some patients. Healing took place in about 3 weeks with thickening and hyperpigmentation of the involved skin. Severe conjunctivitis developed in one patient. Pyrexia (with or without chills) was frequent and usually occurred several hours after the administration of bleomycin. These symptoms usually responded to oral treatment with diphenhydramine hydrochloride (Benadryl) at a dose of 50 mg given three times daily. In one patient treated with a total dose of 280 mg of bleomycin a diffuse, mixed interstitial and alveolar infiltrate associated with significant dyspnea developed throughout both lung fields, contributing to the patient's death.

TABLE 1.—Toxic reactions to bleomycin therapy in 16 patients

Toxic reaction	No. of patients
Nausea	15
Vomiting	10
Stomatitis	14
Dermatitis	12
Alopecia	8
Pyrexia (body temperature > 100 F)	13
Chills	10
Pulmonary infiltration	1
Thrombocytopenia (< 150,000 platelets/mm³)	2

Hematologic toxicity was limited to thrombocytopenia in two patients who had nadir counts of 110,000 and 40,000 platelets/mm³ 20 and 22 days, respectively, after the start of therapy. Although proteinuria occurred in four patients, no significant variations in the serum creatinine levels were observed. In five patients serum alkaline phosphatase was found to have increased to abnormal levels during therapy or at reevaluation. In three patients the increase coincided with other objective evidence of progressive liver metastasis, but in two patients it could not be attributed with certainty to tumor involvement. No changes were observed in serum bilirubin and SGOT levels or in daily blood pressure or pulse recordings during therapy.

Objective Response

Our criteria for an objective response are: (*a*) a decrease of at least 50% in the product of the longest perpendicular diameters of the most clearly measurable area of known malignant disease or, if malignant hepatomegaly is the indicator, a decrease of 30% in the sum of measurements below the xyphoid process and both costal margins at the midclavicular lines during quiet respiration; (*b*) no increase in the size of other areas of malignant disease; and (*c*) no new areas of disease.

None of the 14 patients with cancer of the esophagus had either an objective response or symptomatic improvement. Four patients remained objectively stable but in the remaining ten patients progressive disease was evident. One of the two patients with squamous cell carcinoma of the pancreas, however, had a complete objective response with striking symptomatic improvement.

COMMENT

The lack of any objective or symptomatic response in the 14 consecutive patients in our study, the minimal response rate in the previous cumulative experience in the United

States, and the transient nature of the responses in European reports all indicate that it is very unlikely that treatment with bleomycin alone will assume a significant clinical role in the therapy for squamous cell carcinoma of the esophagus.

The fact that an objective response was achieved in one of two patients with squamous cell carcinoma of the pancreas raises the question of the potential effectiveness of bleomycin for tumors in this anatomical location and warrants further study. We have not found other reports of bleomycin therapy for this rare neoplasm.

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Further Experience With Hexamethylmelamine (NSC-13875) in the Treatment of Carcinoma of the Cervix [1,2,3]

David C. Stolinsky and Joseph R. Bateman [4,5]

In a previous report (1) of a trial of hexamethylmelamine (HMM) in the treatment of 115 patients who had a variety of malignant neoplasms, we noted partial responses in four of ten patients with carcinoma of the cervix uteri. An extension of the previous study, restricted to patients with carcinoma of the cervix, is described in the present report.

METHODS

Patient Selection

Patients with histologically verified carcinoma of the cervix that was not amenable to surgical or radiation therapy were included in the study. Patients were required to have accurately measurable parameters of disease; those patients manifesting only vague pelvic masses or frozen pelvis were ineligible for the study. Patients who had received prior therapy with cytotoxic agents or irradiation were eligible provided that at least 4 weeks had elapsed since cessation of such therapy. Ability to retain oral medication was required, but there was no requirement of minimum performance status. Patients with a serum creatinine value above 2.0 mg/100 ml or a total bilirubin value over 2.0 mg/100 ml were ineligible. Minimum hematologic limits required a white blood cell count (wbc) of at least 4500 cells/mm^3 and a platelet count of at least 150,000 platelets/mm^3. Informed consent was obtained from each patient in writing.

Clinical Evaluation

The following pretreatment observations were obtained: complete history and physical examination which included bidimensional tumor measurement; complete blood cell count (cbc) including platelet count; bone marrow aspiration in most cases; urinalysis; serum urea nitrogen or creatinine, uric acid, prothrombin time, total bilirubin, alkaline phosphatase, glutamic pyruvic transaminase, and glucose determinations; and chest and skeletal roentgenograms. The physical examination and cbc were repeated at least weekly while the drug was administered; serum chemical determinations were repeated at intervals of 2–4 weeks; abnormal roentgenograms of the chest were repeated monthly and those of the skeleton were repeated every 2 months. In the assessment of response, the products of the perpendicular diameters of each measurable tumor were added together.

Drug Administration

HMM was supplied as 50- and 100-mg capsules of the micronized material. It was administered daily after meals in four equal doses for 21 days at a dose level of 12 mg/kg/day. After a 4-week rest period, the course was repeated if the tumor had responded. Antiemetics were used as needed. No other form of antitumor therapy was allowed dur-

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[2] Supported in part by Public Health Service grant CA-05186 from the National Cancer Institute (NCI), National Institutes of Health (NIH), Department of Health, Education, and Welfare.

[3] Hexamethylmelamine: CAS reg. No. 645-05-6. Supplied by Drug Research and Development, Division of Cancer Treatment, NCI.

[4] The following members of the Western Cancer Study Group participated in this study: *J. Bateman, D. Bogdon, G. Hum, L. Irwin,* and *D. Stolinsky* (University of Southern California School of Medicine, and John Wesley Hospital, Los Angeles); *R. Hestorff* (Kaiser Foundation Hospital, Oakland, Calif); *A. Glass* (Kaiser Foundation Hospital, Portland, Ore); *H. Kimball* (Memorial Hospital, Phoenix, Ariz); *J. McCracken* (Madigan General Hospital, Tacoma, Wash); *R. Pugh* (Ellis Fischel State Cancer Hospital, Columbia, Mo); *F. Bull* (University of Michigan, Ann Arbor); *M. Corder* (Letterman General Hospital, San Francisco, Calif); *L. Sadoff* (Kaiser Foundation Hospital, Los Angeles, Calif).

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ing the period of the study. Therapy was reduced or withheld if indicated by the appearance of toxic effects.

RESULTS

Thirty-five patients were treated with HMM. One patient lacked measurable disease parameters and was ineligible for the study. The remaining 34 patients were all considered evaluable for response and toxic effects. However, only 29 patients completed 1 week of therapy and 21 completed a 3-week course. Patients failed to complete 3 weeks of therapy for the following reasons: seven patients died of disease within 3 weeks of the start of therapy; four were unable to tolerate HMM because of drug-induced nausea; two were lost to followup; and one became too ill to take oral medication.

Responses

A partial response was defined as at least a 50% decrease in measurable tumors for a period of at least 1 month with associated symptomatic benefit. Eight partial responses were observed. Responses began at a median of 22 days after the start of therapy (range: 12–36 days) and at a median cumulative dose of 167 mg/kg (range: 102–253 mg/kg). The responses lasted a median of 3.5 months (range: 1.5–8.0 months). A ninth patient manifested a tumor response that was just short of a 50% decrease but which lasted for 11 months. No other responses were obtained.

Response to therapy was noted in one of four patients with osseous metastases, four of 13 with pulmonary metastases, none of three with hepatic metastases, four of six with lymph node metastases, three of 14 with vaginal metastases, one of four with pelvic metastases, one of five with vesical or rectal involvement, and one of six with unilateral ureteral obstruction. All of the observed responses occurred in patients with squamous cell carcinoma of the cervix. One patient with anaplastic carcinoma and one with adenocarcinoma failed to respond.

All of the eight patients who responded to HMM had received prior irradiation. In addition, one patient had received prior methotrexate, and two had received multiple agents including 5-fluorouracil and alkylating agents.[6] This observation is of interest, because in our prior report (1), no patient who responded to HMM had disease that was known to be resistant to conventional alkylating agents. The median duration of disease from first diagnosis was 13 months for the responders and 21.5 months for the nonresponders. However, the nonresponders did not tend to have more extensive disease than the responders. There was no difference between responders and nonresponders with respect to median age, racial distribution, or incidence of toxic effects.

TABLE 1.—Incidence and severity of the toxic effects of HMM in 34 patients

Toxic effect	Degree*			
	Mild	Moderate	Severe	Extreme
Leukopenia	2	5	2	0
Thrombocytopenia	6	3	0	1
Nausea, vomiting	8	11	6	1
Depression	1	2	2	0
Neuropathy	1	3	0	0
Dermatitis	1	0	0	0
Alopecia	3	0	0	0

*See text, Toxic Effects, for definitions of degrees.

Toxic Effects

The incidence and severity of the toxic effects of HMM are listed in table 1. Leukopenia was classified as mild (3000–3900 cells/mm^3), moderate (2000–2900 cells/mm^3), severe (1000–1900 cells/mm^3), and extreme (< 1000 cells/mm^3). Thrombocytopenia was classified as mild (100,000–149,000 platelets/mm^3), moderate (60,000–99,000 platelets/mm^3), severe (35,000–59,000 platelets/mm^3), and extreme (< 35,000 platelets/mm^3). No deaths were attributable to therapy. Hematologic toxic effects occurred in a minority of patients and were rarely of severe degree.

In contrast, nausea and vomiting occurred in most patients and were frequently dose-limiting. In addition to the four patients who

[6] Methotrexate: NSC-740; CAS reg. No. 59-05-2; glutamic acid, *N*-[*p*-[[(2,4-diamino-6-pteridinyl)methyl]-methylamino]benzoyl]-.

5-Fluorouracil: NSC-19893; CAS reg. No. 51-21-8; 5-FU.

were unable to complete one course of therapy because of nausea, 13 patients required significant dose reductions because of nausea. Nausea was only partially controlled by antiemetics; many patients required constant encouragement to continue therapy.

Depression of mood was reported in our previous study (1) and was observed in five patients in the present study. In no patient was mood depression dose-limiting. Symptoms of a mild peripheral neuropathy were reported by four patients. In each of these four patients, the neurologic manifestations subsided when therapy was discontinued. A drug-related dermatitis developed in one patient and alopecia developed in three patients. No hepatic or renal toxic effects were noted.

DISCUSSION

Carcinoma of the cervix is a common tumor and one which is considered relatively resistant to chemotherapy. Reported response rates (2) have averaged 20% for methotrexate, 21% for 5-FU, and 19% for cyclophosphamide.[7] The results obtained with HMM in the present study are somewhat encouraging despite the relatively small number of patients treated. However, the agent causes nausea and vomiting in most patients, and these effects can be quite severe. A reduced dose of HMM was tolerated by some patients who had been unable to tolerate a full dose. Accordingly, it would be of interest to combine HMM at reduced doses with another agent that has shown antitumor activity against carcinoma of the cervix.

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[7] Cyclophosphamide: NSC-26271; CAS reg. No. 6055-19-2; 2*H*-1,3,2-oxazaphosphorine, 2-[bis(2-chloroethyl)-amino]tetrahydro-, 2-oxide, monohydrate.

Modification of Transplantability and Tumor Growth Following Treatment of L1210 Leukemia and TA3Ha Cells With Macromomycin (NSC-170105) [1,2]

Muriel M. Lippman and Betty J. Abbott [3]

Macromomycin (MCR), a protein preparation originally obtained from cultures of *Streptomyces macromyceticus* by Chimura (1), has been shown to be effective in preventing cell division in culture (2) [4] and in prolonging the life of tumor-bearing animals treated with the preparation (2). MCR adsorbs to the cell surface, and it is thought that its action on cells is mediated through this adsorption (2). It has been suggested that the adsorption of macromolecules to cell surfaces would be a means of increasing the immunogenicity of tumor cells (3). If an agent could block cell division and simultaneously increase the immunogenicity of tumor cells, that agent would not only exert a direct chemotherapeutic effect but would also enlist host defenses in elimination of the tumor. The combination of these two modalities should be more effective than cytostatic or cytotoxic effects alone. This communication demonstrates that MCR treatment of tumor cells prior to their transplant reduces the ability of these cells to produce tumors in hosts with histocompatibility barriers otherwise too weak to effect rejection. It is further demonstrated that this is accomplished with the same cells which produce tumors in totally syngeneic hosts, thereby ruling out loss of viability of treated cells as an explanation for the observed phenomenon.

TA3Ha TUMOR SYSTEM

The TA3 mammary adenocarcinoma arose spontaneously in 1949 in an A/Ha mouse in the laboratory of Dr. T. Hauschka; it was subsequently converted to ascites form. The TA3Ha subline which arose spontaneously from the parent tumor transgresses histocompatibility barriers and produces 100% mortality in a variety of hosts with tumor (4), including the BDF_1 mouse used in these experiments. The tumor is readily maintained in suspension culture in RPMI 1630 medium supplemented with 10% fetal calf serum. It is also transplantable back to the host animals with some increase in survival time in all hosts due to passage in culture. In the experiment described here, cultured TA3Ha cells in their 32nd culture passage were used at an intraperitoneal (ip) inoculum level of 10^5 cells. MCR was added to the suspension cultures in a concentration of 1.0 μg/ml, a level shown elsewhere [4] to be cytostatic but not cytotoxic after 24 hours in culture. The cells were washed free of culture medium and were injected into host animals. A single source of MCR-treated or control cells was used for injection into both the A/He and BDF_1 hosts. In A/He hosts the median survival time (MST) was 6 days longer for animals that received MCR-treated cells compared to animals that received cultured control cells. However, the BDF_1 hosts who received MCR-treated cells uniformly rejected the tumor, giving 100% tumor-free survivors beyond 120 days (table 1). These animals are being retained for subsequent challenge with TA3Ha cells to determine whether they are immunized. It has been previously shown (5) that weak immunogenicity rather than tumor cell resistance is responsible for the ability of the TA3Ha tumor to grow in BDF_1 mice. It seems likely that an augmented immuno-

[1] Received July 30, 1973; revised Sept 28, 1973; accepted Oct 1, 1973.

[2] Macromomycin: CAS reg. No. 12634-34-3. Supplied by Drug Research and Development (DR&D), Division of Cancer Treatment (DCT), National Cancer Institute (NCI).

[3] Drug Evaluation Branch, DR&D, DCT, NCI, Bethesda, Md.

[4] Lippman MM, and Bono V. In vitro studies on macromomycin (NSC-170105) cell division blockade at G_2 at non-cytotoxic concentrations. In preparation.

TABLE 1.—Effect of MCR treatment of cultured TA3Ha cells on subsequent tumor growth in hosts with differing degrees of histocompatibility

10^6 TA3Ha cells	A/He mice			BDF_1 mice		
	MST	T/C%	No. of tumor-free survivors/ total No. treated	MST	T/C%	No. of tumor-free survivors/ total No. treated
Not cultured	9.0		0/>100	16.0		0/>100
Cultured control	13.0		0/10	18.0		0/10
MCR-treated* cultured cells	19.0	146	0/10	>120	>660	10/10

*MCR added to cultured cells 24 hours prior to inoculation in concentration of 1 μg/ml. Viability = 100%.

genicity is responsible for the rejection of the cells in BDF_1 mice, while such an increase in immunogenicity did not reach a critical level in the syngeneic host.

L1210 LEUKEMIA TUMOR SYSTEM

In order to determine whether the same result could be obtained with a lesser degree of histoincompatibility, the L1210 tumor, which is syngeneic in the DBA/2 mouse but also grows very well in the hybrid BDF_1 host, was tested.

Suspension-cultured L1210 cells grown in RPMI 1630 medium plus 10% fetal calf serum were accordingly treated for 24 hours with 1 μg/ml of MCR. The cells were injected into either DBA/2 or BDF_1 mice. Three alternative procedures were followed: (*a*) the animals were given no further treatment, (*b*) the animals were given injections of saline on Days 2,4,6, and 8, or (*c*) the animals were given injections of MCR (8 mg/kg) on Days 2,4,6, and 8. Ten animals were used in each group. Table 2 summarizes the results of this study. It can be seen that a significant increase in survival time was obtained when cultured cells were treated with cytostatic concentrations of MCR prior to inoculation into BDF_1 mice, but such increases did not occur in the syngeneic DBA/2 mice. The same type of differential activity, but of lesser magnitude, is, therefore, observed with L1210 cells in syngeneic versus

TABLE 2.—Effect of pretreatment of cells with MCR on survival time of DBA/2 and BDF_1 mice with and without subsequent treatments

	DBA/2 mice			BDF_1 mice		
	MST (range)	T/C%	No. of tumor-free survivors/ total No. treated	MST (range)	T/C%	No. of tumor-free survivors/ total No. treated
MCR-treated cells only	16.5 (14–28)	91	1/10	38.0 (32–45)	133	1/10
Control cells only	18.0 (16–22)		0/6	28.5 (20–43)		0/10
MCR-treated cells + saline (ip)*	28.0 (15–48)	155	0/10	60.0 (33–>60)	>206	6/10
Control cells + saline (ip)*	18.0 (15–28)		0/10	29.0 (17–35)		0/10
MCR-treated cells + MCR (ip)*	27.0 (24–33)	142	0/10	60.0 (32–>60)	>187	5/10
Control cells + MCR (ip)*	19.0 (17–24)		0/10	32.0 (24–>60)		1/10

*Saline given ip or MCR given ip on Days 2, 4, 6, and 8.

hybrid hosts as with TA3Ha cells in syngeneic versus allogeneic hosts. Followup injection with sterile saline was as effective as followup injection with MCR in augmenting the response in both DBA/2 and BDF_1 hosts. Thus, apparently nonspecific stimuli were only able to prolong life by 50% in DBA/2 hosts, while the BDF_1 mice survived much longer and over 50% of the animals remained tumor-free. The unexpected response to followup saline injection may be explained in several ways. One such explanation is that of extensive macrophage mobilization resulting from repeated needle puncture or saline injection-produced nonspecific augmentation of the immune processes. The ability of saline injection to effect such a macrophage increase has been demonstrated by Hartveit (6). In addition to the Trypan Blue exclusion test which indicated 100% viability of the cells used in these studies, a parallel study with trypsinization of control and MCR-treated cells completely restored the reproductive capabilities of the treated cells verifying the fact of their viability.

In contrast to these results on cultured cells, direct ip injection of MCR, starting on Day 1, into BDF_1 mice carrying L1210 tumors, prolonged survival time with a treated/control (T/C) value not greater than 138% at doses up to 48 mg/kg, but such treatment did not result in any tumor-free survivors. Similarly, L1210 cells taken from BDF_1 hosts and incubated for varying lengths of time up to 5.5 hours in MCR also failed to produce tumor-free survivors but did significantly increase survival time beyond that obtained by direct ip injection. Data on these findings will be fully presented elsewhere.

It appears that the increased immunogenicity which accompanies prolonged culture of cells plus some modest degree of host histoincompatibility as well as the MCR adsorption are required for maximal effectiveness.

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ANNOUNCEMENTS

1st European Congress on Thermography

The European Thermographic Association, in collaboration with the American Thermographic Society, announces the 1st European Congress on Thermography, to be held in Amsterdam, The Netherlands, on June 17-20, 1974. The scientific program will include invited lectures, specialized papers, audiovisual displays, and scientific and technical exhibitions. For further information write to the Secretariat, 1st European Congress on Thermography, Organisatie Bureau Amsterdam N.V., PO Box 7205, Amsterdam, The Netherlands.

Postdoctoral Fellowships in Pediatric Hematology-Oncology

Childrens Hospital of Los Angeles, Department of Pediatrics, University of Southern California School of Medicine

The Division of Hematology has a limited number of fellowship openings in pediatric hematology-oncology for physicians who have had at least 2 years of postgraduate pediatric training or for individuals who hold a PhD degree in the biomedical sciences. Candidates must be US citizens or have immigration visas. Physicians will require licensure in California. The fellowship program is designed primarily to train academicians and provides both clinical and research experience.

The staff of the Division of Hematology consists of eight full-time hematologist-oncologists and two biochemists. A physician-fellow will devote most of his first year to working in clinical hematology-oncology, and his second year to a research program. Active research programs in oncology, coagulation, experimental hematology, cytogenetics, immunology, pharmacology, and cellular and molecular biology will be available to PhD and MD fellows. Trainees will be given a broad educational program which includes principles of therapeutics, cytodiagnosis, pharmacology, nuclear medicine, relevant topics in cellular biochemistry and physiology, and statistical and experimental design. The program will be tailored to the career goals of each individual candidate.

A third year fellowship is available. The first year of fellowship is accredited by the American Board of Pediatrics as a third year of pediatric training.

Candidates interested in fellowship opportunities should write to: Carol B. Hyman, MD, Director, Fellowship Training, or Myron Karon, MD, Head, Division of Hematology, Childrens Hospital of Los Angeles, 4650 Sunset Blvd, Los Angeles, Calif 90027.

New Drug Seminar

The Division of Cancer Treatment, National Cancer Institute, is planning its fourth New Drug Seminar to be held on February 15, 1974, in the Jack Masur Auditorium on the NIH reservation in Bethesda, Md. The subject of the seminar will be bleomycin (Blenoxane), a new antitumor antibiotic developed originally in Japan and recently approved for usage in the United States by the Food and Drug Administration. Bleomycin is approved for usage in malignant lymphomas, squamous cell tumors of the head and neck region, and testicular neoplasms. The seminar will cover the drug's initial development, mechanism of action, initial clinical evaluation in Japan, as well as many reports on the American experience, including a detailed look at the pulmonary toxicity problem. The seminar should be of interest to oncologists and hematologists, internists, and other physicians with interests in the treatment of cancer. For further information please contact: Dr. C. Gordon Zubrod, Director, Division of Cancer Treatment, National Cancer Institute, National Institutes of Health, Bldg 31, Rm 3A52, Bethesda, Md 20014.

Proposed Interdisciplinary Cell Kinetic Society

An organizational meeting for a proposed interdisciplinary cell kinetic society is being planned for late March 1974, in Houston, Texas. Further information can be obtained by writing to Lewis M. Schiffer, MD, Allegheny General Hospital, 320 E North Ave, Pittsburgh, Pa 15212.

Combined Modality Therapy of Cancer

The Cancer Clinical Investigation Review Committee of the National Cancer Institute is sponsoring an Information Symposium on Combined Modality Therapy of Cancer on February 7, 8, and 9, 1974. The meeting will be held at The Denver Hilton, 1550 Court Pl, Denver, Colorado 80202.

The object of this Symposium is to acquaint physicians with recent developments in the treatment of cancer using various combinations of surgery, radiotherapy, and chemotherapy. The clinician is faced with the responsibility of informing himself about and incorporating these new principles into the management of his patients.

The topics to be discussed include (1) experimental studies which provide models for the clinical approaches; (2) established clinical methods of improving cancer therapy results using combination treatment approaches; and (3) subjects related to the implementation of combined modality techniques in community practice. The speakers will be clinical investigators who have made outstanding contributions to the improved treatment of cancer using integrated combination therapy methods.

Practicing physicians, house staff, and medical students are invited to attend. For a detailed program and further information write to: Mrs. Jeannette Steinbraker, National Cancer Institute, Rm 10A03 Westwood Bldg, Bethesda, Md 20016.

Seventh National Cancer Conference Proceedings

The SeventhNational Cancer Conference Proceedings has been published by the J. B. Lippincott Company, East Washington Square, Philadelphia, Pa 19105. This 968-page book may be purchased through the Medical Division of the J. B. Lippincott Company at a cost of $17.00.

Guidelines for Comprehensive Nursing Care in Cancer

The Memorial Sloan-Kettering Cancer Center announces the availability of *Guidelines for Comprehensive Nursing Care in Cancer*, a report of a series of continuing education seminars in the care of the patient with cancer. The book is published by the Springer Publishing Company, Inc., 200 Park Ave South, New York, NY 10003, at a cost of $7.50.

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